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J. clin. Path. (1948), **1**, 269.

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN A ROUTINE LABORATORY

BY

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(RECEIVED FOR PUBLICATION, JUNE 25, 1948)

The quantitative measurements made in a haematological laboratory assess the magnitude of deviations from the normal, and successive readings indicate either the response to treatment or the natural progress of the disease. These figures can be of little value unless the range of chance variation that several laboratory workers may record in one blood sample is less than the difference that the clinician requires to distinguish. It has been usual for the standard of precision of a method to be set by one expert, whose results, while measuring the minimum error, may bear little relation to ordinary routine practice. In the present study an attempt was made to assess the errors of some common haematological methods as they are used as a routine in laboratories. Those taking part in the experiments were laboratory technicians and doctors of average skill, who carried out the investigations carefully; any gross technical inaccuracies can be excluded. It was hoped that the experiments might suggest which techniques were likely to give the most generally reliable results. The methods studied were: haemoglobin estimation, red cell count, and haematocrit, from which the errors of the colour index, mean corpuscular volume, and mean corpuscular haemoglobin concentration could be calculated. Other methods included were the mean cell diameter derived from the Price-Jones curve, reticulocyte count, platelet count, whole blood coagulation, and red cell fragility. The white cell count was not considered because, since the work of Berkson and others (1935, 1940) it is widely recognized that this investigation has a large error.

Technique*

Red cell counts.—These were made by diluting the blood in Hayem's fluid to 1/200 and counting eighty small squares in a Neubauer counting-chamber.

Haemoglobin.—This was estimated by two methods:

1. The Haldane method, using a standard calibrated by the National Physical Laboratory. The tubes were matched against a uniformly illuminated screen. 14.7 g. haemoglobin were taken to represent 100 per cent (King and others, 1947).

2. A neutral grey photometer method using an instrument designed by Duffie (1945). In this method 0.02 ml. of blood was pipetted into approximately 2 ml. of distilled water in a cylindrical tube of standard diameter. The oxyhaemoglobin content was measured in terms of the optical density of the solution in comparison with a neutral grey wedge calibrated in grammes of haemoglobin, 14.7 g. being taken to represent 100 per cent.

Packed cell volume.—This was measured in Wintrobe's tubes, using oxalated venous blood in which the proportion of oxalate recommended by Wintrobe (1946) was used.

Price-Jones curves.—These were constructed using a projecting microscope and a high-power objective in a dry system giving a magnification on the screen of 2,000. The cells were measured directly, and each mean cell diameter was recorded with no preliminary outlining in pencil.

Platelets.—Platelets were counted by two methods:

1. Dameshek's method (1932). In this method the platelets in capillary blood, stained with brilliant

* The determinations of red cell count, haemoglobin, packed cell volume, and mean cell diameter were made from the same oxalated venous blood sample. The reticulocyte counts and platelet counts were made from capillary blood.

TABLE I

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN THE ROUTINE LABORATORY. (THE RELEVANT ERRORS ARE LISTED UNDER THE HEADINGS STANDARD DEVIATION AND COEFFICIENT OF VARIATION: THE COLUMN HEADED PROBABLE MINIMUM ERROR SUGGESTS THE LOWEST LIMITS OF ERROR WHICH COULD BE ATTAINED BY ONE HIGHLY SKILLED OBSERVER)

Investigation	No. of observations	Mean	Standard deviation	Coefficient of variation (%)	Significant diff. $\pm 2\sqrt{2s^2}$	Range $\pm$ 2s	Probable minimum error	Some previous estimates of error	Some previous estimates of the normal range
Haemoglobin, Haldane method	50	97	5.1%	5.2	14.4%	87–107%	2–3%	2–13% Macfarlane (1945)	84–116% Price-Jones *et l.* (1935)
Haemoblobin, neutral grey photometer	50	97%	2.9%	3.0	8.2%	91–103%	2–3%	4.3% Macfarlane *et al.* (1948)	
Red cell count	50	4.39 millions	0.415 millions	9.5	1.17 millions	3.56–5.22 millions	7.8%	7.8% Berkson *et al.* (1935, 1940) 1.4% Wintrobe (1946)	4.5–6.4 millions Price-Jones *et al.*
Haematocrit, Wintrobe's method	50	45.5%	0.4%	1 0	1.1%	44.7–46.3%	0.5%	0.5% Wintrobe (1934)	
Haematocrit, micro-method ..	20	45.4%	0.9%	2.0	2.54%	43.6–47.2%	0.5%	0.5% Miller (1938–9)	
Colour index, Haldane method	50	1.12	0.12	10.7	0.339	0.87–1.36	8.10%		0.85–1.15 Osgood (1935)
Colour index, neutral grey photometer	50	1.12	0.11	9.9	0.311	0.90–1.34	8–10%		
Mean corpuscular haemoglobin concentration, Haldane method	50	31.44%	1.59%	5.0	4.49%	28.26–34.62%	2–3%	2.08% Wintrobe (1931–2)	28.17–38% (Various authors)
Mean corpuscular haemoglobin concentration, neutral grey photometer	50	31.44%	0.97%	3.1	2.74%	29.5–33.4%	2–3%		
Mean corpuscular volume ..	50	104.6cμ	10.1cμ	9.6	28.6cμ	84.4–124.8cμ	7–9%	1.6% Wintrobe (1931–2)	

TABLE I (*continued*)

THE ERRORS OF SOME HAEMATOLOGICAL METHODS AS THEY ARE USED IN THE ROUTINE LABORATORY. (THE RELEVANT ERRORS ARE LISTED UNDER THE HEADING: STANDARD DEVIATION AND COEFFICIENT OF VARIATION: THE COLUMN HEADED PROBABLE MINIMUM ERROR SUGGESTS THE LOWEST LIMITS OF ERROR WHICH COULD BE ATTAINED BY ONE HIGHLY SKILLED OBSERVER)

Investigation	No. of observations	Mean	Standard deviation	Coefficient of variation (%)	Significant diff. $\pm 2\sqrt{2s^2}$	Range $\pm$ 2s	Probable minimum error	Some previous estimates of error	Some previous estimates of the normal range
Mean cell diameter measuring 100 cells on one slide ..	25	7.04μ	0.231μ	3.3	0.65μ	6.58–7.50	0.07μ	0.073μ Price-Jones (1933) 0.072μ Mogensen (1938)	6.815–7.492μ Price-Jones (1933)
Mean cell diameter measuring 500 cells on one slide ..	Calculated	7.04μ	0.229μ	3.25	0.647μ	6.58–7.50	0.07μ		
Mean cell diameter, 5 observers measuring 100 cells on five slides (500 cells in all)	Calculated	7.04μ	0.09μ	1.28	0.254μ	6.86–7.22			
Reticulocytes, dry slide method	18	6%	2.4%	40	6.8%	1.2–10.8%		0.54% with 15% reticulocytes 0.44% with 2% reticulocytes Plum (1942)	
Reticulocytes, coverslip method	18	7.2%	3.1%	43	8.7%	1.0–13.4%			
Platelets, Dameshek's method ..	25	365 thousands	151 thousands	41	427 thousands	63–667 thousands	10–15%	3.8% Dameshek (1932)	
Platelets, Lempert's method ..	25	246 thousands	56 thousands	23	158 thousands	134–358 thousands	10–15%	1.8% Olef (1935)	
Whole blood coagulation, Lee and White's method	40	4.8 min.	0.226 min.	4.7	0.64	4.35–5.25 min.			
Whole blood coagulation, Dale and Laidlaw's method	40	1.5 min.	0.228 min.	15.2	0.63 min.	1.04–1.96 min.			
Whole blood coagulation, capillary tube method	40	2.0 min.	0.276 min.	13.8	0.78 min.	1.45–2.55 min.			

cresyl blue, are counted with an oil immersion objective in relation to 1,000 red cells and the total number of platelets are assessed from a separate red cell count.

2. Lempert's (1935) modification of Kristenson's method. In this method the platelets are counted directly in a counting chamber after dilution to 1/20 with a freshly prepared solution containing brilliant cresyl blue to render the platelets more readily visible, and urea to haemolyse the red cells.

Percentage of reticulocytes.—The counts were made by two methods:

1. A drop of a saturated solution of brilliant cresyl blue in alcohol was allowed to dry on a perfectly clean slide, and a small drop of capillary blood collected on a clean coverslip was inverted over the dye to form a very thin film. The preparations were allowed to stand for half an hour for staining to occur.

2. Five drops of a mixture of one part of a saturated solution of brilliant cresyl blue in alcohol, and 4 parts 1.0 per cent sodium citrate in normal saline were placed in a small tube. To this was added one drop of blood. If these proportions are carefully observed the red cells will not crenate. The tubes were shaken and allowed to stand for twenty minutes at 37° C. The contents of the tubes were then thoroughly mixed, and films made on clean slides were stained with Leishman's stain. In both methods the reticulocytes were clearly visible, but in the coverslip method it was not always easy to prepare films in which the red cells were sufficiently separated for them to be counted with accuracy.

Whole blood coagulation.—This was measured by three methods:

1. Lee and White (1913). Venous blood was collected in a paraffined syringe and 1 ml. placed in each of four small test tubes of uniform bore which were kept at 37° C. The coagulation time was measured from the time of filling the syringe.

2. Dale and Laidlaw (1911–12). Small tubes of wide capillary bore, each containing a freely mobile lead shot, were prepared. These were rapidly filled with capillary blood and, held by forceps, were immersed in water at 37° C. The tube was inverted at intervals and the time at which the lead shot failed to move was recorded.

3. Capillary blood was taken into a number of fine capillary tubes, the ends of which were then sealed with plasticine. The tubes were kept at 37° C. Pieces of tubing were broken off at intervals until a clot was withdrawn (Sabràzes, 1904).

Red-cell fragility.—This was measured by a method similar to that of Dacie and Vaughan (1938). The saline dilutions differing by 0.02 per cent were made up individually from sodium chloride dried to a constant weight, and each was tested with a standard silver nitrate solution. Heparin was used as anticoagulant, the volume of plasma was adjusted to a normal haematocrit reading, and the blood was oxygenated immediately before use. In the original technique the blood was added to 1 ml. of each saline dilution from a dropping pipette; in this experiment 0.02 ml. amounts were measured with a series of haemoglobin pipettes. After the tubes had stood in the refrigerator the degree of haemolysis was read against a set of dilutions from completely haemolysed blood made from each sample tested.

Results

The total errors for the methods studied, in terms of their standard deviations and coefficients of variations, are shown in Table I.

The experiments were planned to separate by analysis of variance those components of error likely to be of importance in routine practice. Thus, for example in the red cell count, errors due to pipettes and counting-chambers and to differences between observers could be calculated. In whole-blood coagulation on the other hand, where observers are unlikely to disagree, differences between the order of taking the samples on one day and between samples taken on separate days were measured.

The component errors (S_1, S_2, S_3 . . .) are related to the total standard deviation (S_T) by the formula:

$$S_T^2 = S_1^2 + S_2^2 + S_3^2 \text{ (Formula 1)}$$

Any part of the total error not attributed to a specific factor was classed as random variation.

Haemoglobin.—The errors inherent in estimating haemoglobin have been worked out with considerable care by Macfarlane (1945) and Macfarlane and others (1948). Our results confirm these more detailed observations.

In this experiment five observers each made ten readings both by the Haldane method and with the neutral grey photometer, using ten pipettes and a single sample of oxalated blood. From an analysis of variance the errors referable to the observers and the calibration of the pipettes could be calculated. In addition the components of error due to reading the neutral grey photometer, the calibration, and the filling of the pipettes were determined in a series of separate experiments. The error of reading the photometer was measured by making twenty readings from one diluted sample of blood. The error caused by inaccuracy in filling the pipettes combined with the error of reading the photometer was estimated by filling one pipette ten times and reading the results on the photometer. The error due to calibration of pipettes together with those due to filling and to reading the photometer was assessed by filling ten different pipettes and reading the results on the photometer. From these observations the component errors could be calculated, using Formula (1).

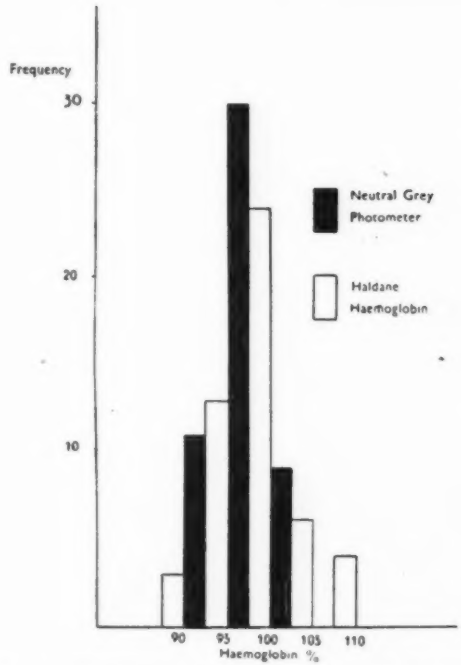

FIG. 1.—Histogram to show the distribution of individual readings for haemoglobin estimation by the Haldane method and using a neutral grey photometer. With each method five observers made ten estimations on one sample of venous blood using ten pipettes.

Fig. 1 shows the distribution of the different values for the haemoglobin obtained by the five observers. In the Haldane method there was a wider dispersion of the readings than was shown by the neutral-grey photometer. In Table II the errors for the two methods obtained by analysis of variance are compared with each other and with those derived from the separate experiments. It will be seen that the greater precision of the neutral-grey photometer results from a reduction in both random variation and the differences between observers.* In addition it will be seen that the random error of the analysis of variance for the photometer method, which necessarily includes that of filling pipettes, is entirely accounted for by this error, which was determined separately. Since the error of the observers and of reading the photometer are both of the order of 1 per cent, the error of the photometer method is probably approaching the minimum for haemoglobin determination.

*Since only five observers took part in the majority of the experiments described, the observer component is seldom determined with great precision.

Red cell count.—The error of the red cell count has been the subject of much controversy. On the one hand Berkson and others (1935, 1940) have shown that the minimum error is of the order of 7.8 per cent, whereas other workers believe that far greater uniformity is possible. Wintrobe (1946) stated: "In spite of the fact that statistical analysis indicates that the error in counting red cells may be as great as 16 per cent even in the hands of trained technicians, it will be found that, with practice, counts agreeing within 200,000 cells per c.mm. can be made." Our results have favoured Berkson and others' estimate of the error, but it is also true that trained technicians can make counts that will agree within 200,000 cells. However, this uniformity cannot be maintained if several skilled workers each make a series of independent counts. It will then be found that their mean values differ more widely than would be expected from chance variation (Biggs and MacMillan, p. 288 of this issue). The "accuracy" of the skilled haematological technician is a product of the method of training. It is usual to set a standard of agreement between repeat counts which must be achieved before a worker's results are considered reliable. Since this standard is invariably one that cannot be reached by accurate

TABLE II

THE COMPONENTS OF ERROR IN HAEMOGLOBIN ESTIMATION EXPRESSED AS A PERCENTAGE OF THE MEAN HAEMOGLOBIN VALUES: BASED ON FIFTY OBSERVATIONS MADE BY FIVE OBSERVERS ON A SINGLE SAMPLE OF BLOOD

Source of error and mean value	Estimation of error by separate experiments (%)	Analysis of variance in the neutral grey photometer method (%)	Analysis of variance for Haldane method (%)
Mean ..	97	97	97
Total error ..	3.3*	3.0	5.2
Calibration of pipettes ..	2.0	1.8	2.4
Filling pipettes	2.2		
Observers ..		1.0	2.8
Reading photometer ..	1.38		
Random error		2.2	3.7

* Excluding the observer component.

counting, the technician learns to count very rapidly and to make continuous unconscious adjustment in a series of counts to ensure that all will agree with the first.

In this experiment five observers made ten red cell counts from the sample of blood used in the haemoglobin determinations, using ten pipettes and ten counting chambers. One red cell pipette was always used with the same counting chamber, and thus the errors due to these two factors were estimated together.

It will be seen from Fig. 2 that the total range of variation was very large, rather larger than that

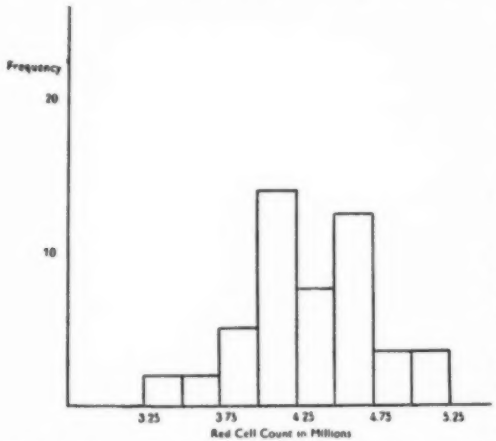

FIG. 2.—Histogram to show the distribution of individual red cell counts. Five observers each made ten estimations from the sample of blood from which the haemoglobin determinations were made, using ten pipettes and ten counting chambers. Each pipette was always used with the same counting chamber.

recorded by Berkson and others. This increase in error is due both to the inclusion of different observers in the experiment and to the fact that, whereas Berkson and others counted the red cells with an electric counter from photographs, in these experiments the cells were counted by eye. In addition it will be seen from Table III that the component errors are rather different. Berkson and others record a larger error for pipettes and counting chambers, while our results show a greater random fluctuation. This discrepancy is due to the different methods used to discover the errors. Berkson and others made a series of separate experiments similar to those described for the errors of the haemoglobin pipette and reading the neutral-grey photometer, while our results were

TABLE III

THE COMPONENTS OF ERROR IN RED CELL COUNTING EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES FOR THE RED CELL COUNT. THE RESULTS OF OUR EXPERIMENTS COMPARED WITH THOSE OF BERKSON AND OTHERS (1940)

Source of error and mean value	Results from analysis of variance in our experiments	Results of Berkson and others' experiments
Mean value ..	4.39 millions per c. mm.	
Total error ..	9.46%	7.8%*
Pipettes	4.26%	4.7%
Counting chambers		4.6%
Observers	3.12%	
Random error ..	7.8%	4.2%

* Excluding the observer component.

obtained by analysis of variance. The figure for random error in our calculations includes the error of filling the pipettes, which was estimated separately by Berkson and others.

The variability of this method, from which repeated counts on the same sample may be equivalent to differences of more than 1,000,000 cells per c.mm. in a 5,000,000 count indicates that small changes in red cell count cannot be measured with reasonable precision.

Packed cell volume.—A previous experiment in which Wintrobe (1934) estimated the error of the haematocrit as 0.5 per cent suggests that this method should give uniform results.

In this experiment each of the five observers filled ten Wintrobe haematocrit tubes with blood from the sample used in the red cell counts and haemoglobin determinations. These were then centrifuged at 3,000 r.p.m. for half an hour. There were no differences to be detected between the tubes. The only error was a small random fluctuation of 0.5 per cent, giving a coefficient of variation of 1 per cent. This method has an almost negligible error and since some of the techniques applicable to capillary blood are also reliable (Miller, 1938–39) the method is well adapted to routine use. The error of a capillary method in use in this laboratory is shown in Table I.

Indices.—The red cell counts, the determinations of haemoglobin, and the packed cell volume were all made on the same sample of blood, and since the relations between the haemoglobin and

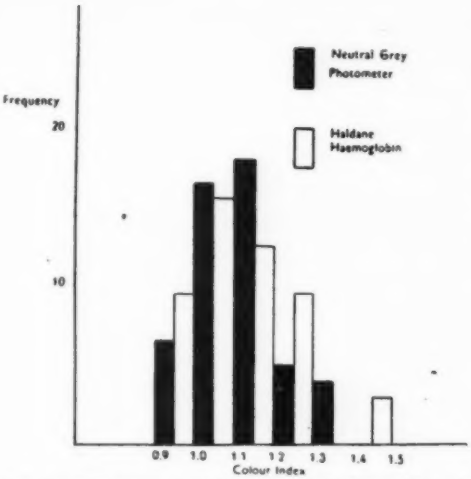

FIG. 3.—Histogram to show the distribution of the calculated values for the colour index using both the Haldane and neutral grey photometer methods. Each haemoglobin estimation was paired at random with a red cell count made by the same observer.

red cell count, the haematocrit and red cell count, and the haematocrit and haemoglobin for normal blood can all be represented by linear regression lines passing through the origin, the errors may be calculated with sufficient accuracy by pairing appropriate values at random and determining the standard deviations of the resulting indices.

The magnitudes of these errors are given in Table I, and the distribution of the individual observations in Figs. 3 to 5. It is obvious that the indices derived from variable observations are unlikely to measure small differences reliably, but the wide range of the colour index and mean corpuscular volume is illuminating. By chance the sample of blood examined gave mean values for the colour index and mean corpuscular volume which are at the upper limit of the normal range 1.12 and 104.6 μ respectively. It will be seen that in a blood sample of this type, encountered not infrequently in routine work, there is a high probability of recording a grossly pathological value. The fact that these exaggerated figures seldom reach the clinician may be attributed to the natural prejudice of technicians and pathologists in favour of reasonable results; a prejudice which leads to the repetition of values incompatible with the appearance of the blood film or grossly different from previous observations on the same patient.

Both of these indices are used to measure differences that are small in relation to their range of chance fluctuation. Neither is therefore of great value as a single observation, though successive readings will give a general impression of any systematic trend over a period of time, such, for example, as may occur during the treatment of pernicious anaemia.

On the other hand the mean corpuscular haemoglobin concentrations showed relatively little variation, and this index, which is well adapted to the study of iron deficiency, could be used more frequently as a routine estimation.

Mean cell diameter derived from the Price-Jones curve.—Both Price-Jones (1933) and Mogensen (1938) have studied the errors inherent in drawing a Price-Jones curve, including the variation in cell size in one subject on repeated measurements from different slides, and the range in normal subjects (Price-Jones, 1933). The error in making repeated observations on one subject was small, but it is usually believed that the technique, in addition to being extremely time-consuming, is of little value except in the hands of highly skilled and experienced observers. Our experiment was undertaken to discover whether relatively unskilled workers could obtain information of any clinical value. Five observers measured a hundred cells from each of five slides taken from the same subject at the same time. The errors referable to differences between observers and those between slides could therefore be calculated.

It will be seen in Fig. 6 that a wide variation occurred. The range of cell size in any one estimation (the standard deviation of one Price-Jones

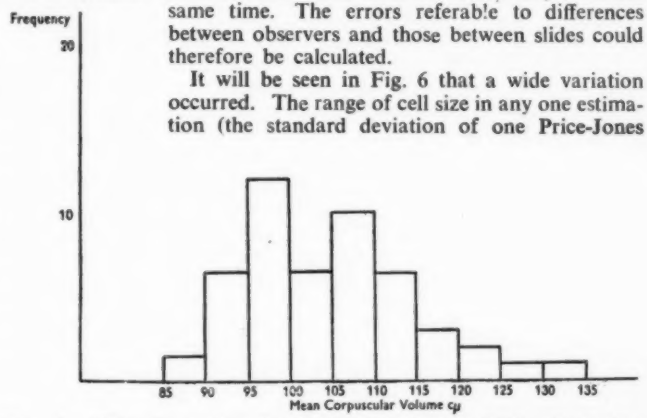

FIG. 4.—Histogram to show the distribution of the calculated values for the mean corpuscular volume. Each of the red cell counts was paired at random with a haematocrit reading made by the same observer.

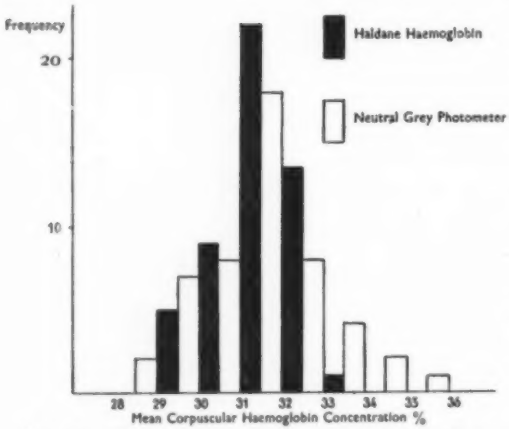

FIG. 5.—Histogram to show the distribution of the calculated values for the mean corpuscular haemoglobin concentration using both the Haldane and neutral grey photometer methods for haemoglobin determination. Each haemoglobin determination was paired at random with a haematocrit reading made by the same observer.

curve) was similar to those of Mogensen and Price-Jones and remained fairly constant throughout. The variation between different slides was also similar to those found by Mogensen and Price-Jones (Table IV). The increased error was due to differences between observers and a component classed as "interaction." Neither Mogensen nor Price-Jones considered the possibility that

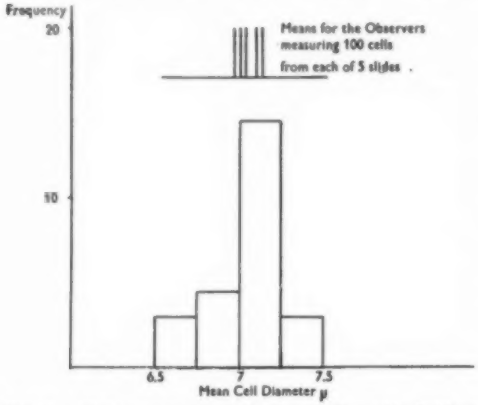

FIG. 6.—Histogram to show the distribution of individual determinations of the mean cell diameter. Five observers measured one hundred cells from each of five slides. The mean values for each observer measuring one hundred cells from the five different slides are shown above.

TABLE IV

THE COMPONENT ERRORS OF DETERMINING THE MEAN CELL DIAMETER FROM PRICE-JONES' CURVE EXPRESSED AS STANDARD DEVIATION IN μ. THE RESULTS OF OUR EXPERIMENTS COMPARED WITH THOSE OF MOGENSEN (1938) AND PRICE-JONES (1933)

Source of error and mean value	Error determined from our experiments	Error assessed by Mogensen	Error assessed by Price-Jones
Mean	7.04	7.44	7.328
Total error ..	0.231		
Error for 1 observer measuring 100 cells on one slide ..	0.143	0.0721	0.0735
Differences between observers ..	0.171		
Differences between slides	0.08	0.06	0.06
Error component classed as "interaction"	0.1265		
Random error per mean of 100 cells	0.044	0.045	0.048

observers might not always agree, a factor of importance in clinical practice.

As was suggested by Mogensen (1938), and more recently by Humble and Belyavin (1948), the only error reduced by increasing the number of cells measured is that due to chance variation in the population of cells selected from a particular slide. In our estimations this error was 0.04 μ which, compared with a total error of 0.231 μ, is negligible, and no appreciably greater precision results from increasing the number of cells measured to 500 (Table I). On the other hand, if the larger errors due to the slides and observers were reduced, the precision would be increased. This can be effected if, for example, five observers each measure a hundred cells from different slides. Since only five hundred cells are measured by the five observers together, there is no increase in the total labour of Price-Jones' original technique; in this way the error is reduced from 0.231 to 0.09 μ. In Fig. 6 the mean values for the five observers' measurements on different slides are compared with the total invariability. Although the error of the mean cell diameter is reduced by this method, a false impression of anisocytosis will follow

systematic differences between observers. This may be avoided if the Price-Jones curve is constructed from the results of one observer.

The error is large compared with those of Price-Jones and Mogensen, but it is small in comparison with those of other investigations that give any indication of cell size (Mean Corpuscular Volume and Colour Index). In addition the Price-Jones curve gives a valuable record of anisocytosis. Again, if highly reproducible results are required, the method by which five observers make measurements on five slides will give a precision approaching that of the experts, even including the observer component. Thus from the view point of precision the Price-Jones curve, even when constructed by the average laboratory worker, will give information of clinical value. The main disadvantage that remains is the length of time occupied by a single observation. However, we did not find that this was excessive. In forty-five minutes one observer may make all necessary measurements and complete the calculations, using a simplified technique such as that described by Humble and Belyavin (1948); no doubt this time could be reduced still further. Thus, if, in a case of pernicious anaemia for example, instead of making repeated observations of the Colour Index and Mean Corpuscular Volume, two Price-Jones curves were drawn, one before and the other following treatment, there would be little total loss of time, and the record of the patient's progress would be more complete and more precisely assessed.

Reticulocyte count.—Plum (1942) and Jacobsen and others (1947) have shown that repeated reticulocyte counts on one sample of blood will agree closely. It is, perhaps, difficult to understand why the errors which they record appear rather smaller than the random selection of 1,000 cells from a binomial distribution would allow. Nevertheless, even acknowledging the full standard deviation to be expected, which is of the order of 1 per cent of reticulocytes for counts from 5 to 10 per cent, it might be hoped that this would prove one of the more reliable routine methods.

In this experiment nine observers counted the number of reticulocytes in five hundred red cells from one sample of venous blood. They made two preparations by each of the methods, and thus made four counts in all. In this way any difference between the observers and between the two methods could be measured. No consistent difference between the methods was found.

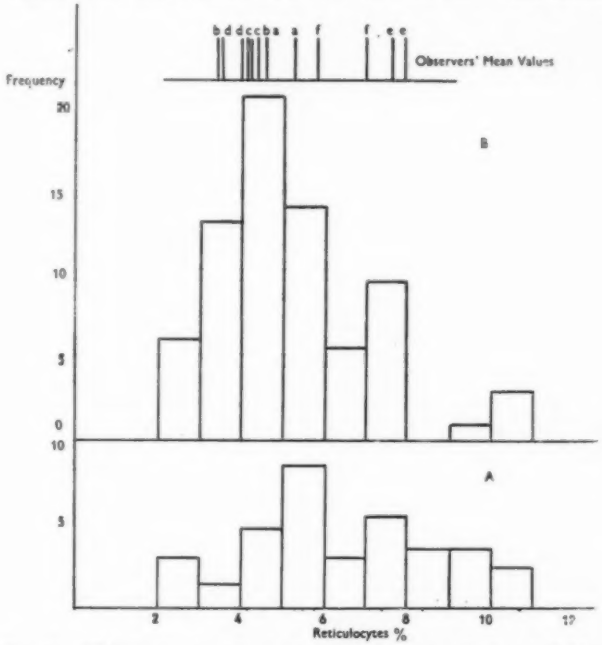

FIG. 7.—Histograms to show the distributions of the individual reticulocyte counts. The two parts of the diagram refer to different experiments (see text). In Fig. 7b the mean values obtained by the observers by the two methods of counting are indicated.

From Fig. 7a it will be seen that a large range of variation occurred, very much larger than should result from random selection from a binominal distribution. In both methods there was a considerable difference between the results of different observers (Table V). In the coverslip method there was also a big random error, probably caused by difficulty in accurate counting on films in which the cells were not well separated. In the slide method the two counts made by each observer agreed fairly well and thus the random error was small. Of these two, the slide method was therefore preferable.

The large observer error was, however, surprising, and an experiment was designed to decide whether this was due to differences between the preparations made by the observers or to difficulty in the recognition of reticulocytes. Six experienced

TABLE V

THE COMPONENT ERRORS OF THE RETICULOCYTE COUNT EXPRESSED AS STANDARD DEVIATION IN PERCENTAGE OF RETICULOCYTE

Source of error and mean value	Coverslip method (%)	Dry slide method (%)
Mean	7.2	6.0
Total standard deviation	3.1	2.4
Error due to observers	2.2	2.2
Random error ..	2.2	0.9

observers made counts from six slides made at the same time from one stained drop of blood. Inaccuracy in enumeration was avoided by reducing the field to contain between ten and thirty cells. The errors due to uneven distribution on one slide was minimized by making counts not only by recording the number of reticulocytes in five hundred red cells but also by a method of inverse sampling suggested by Woolf (1948). A hundred reticulocytes were counted and the number of fields passed over before this total was reached was recorded. The number of red cells per field was calculated from a random sample of 200–300 red cells. In this way the number of reticulocytes in approximately 2,000 red cells was assessed.

From Fig. 7b it will be seen that there was slightly more tendency for the values to be grouped than in the original experiment, but the variation was still large. The individual observers obtained fairly consistent results with figures ranging from 2.4 to 4.8 per cent, 3.2 to 4.2 per cent, 2.6 to 6.4 per cent, 4.1 to 6.6 per cent, 4.4 to 7.2 per cent, and 6.4 to 9.8 per cent, but clearly the divergence of opinion between them was wide. A further study of this observer difference has shown that the number of reticulocytes seen is controlled both by the eyesight of the observer and by the magnification of the microscope used. Since this is an error that cannot readily be eliminated, the best results for clinical purposes will be obtained if the same observer makes all the counts on any one patient.

Platelet count.—The indirect and direct platelet counts, of which Dameshek's and Lempert's methods were chosen, are known to give different figures for the normal number of platelets. The indirect-methods, in which the platelets are counted in proportion to the number of red cells and the total deduced from a separate red cell count, give the higher results, and therefore have the advantage of preserving a greater proportion of the platelets. From the techniques involved neither of these methods can be precise. In Lempert's method the error must include those due to pipetting and the uneven distribution of platelets. The minimum error of this method is likely to exceed that of the red cell count because fewer particles are counted. In the indirect methods the error cannot be reduced below that of counting red cells, combined with the error due to uneven distribution of the platelets on the slide preparations. In addition to these the scope for divergence of opinion between observers is great. According to Aggeler and others (1946), ". . . there may be wide variations in the results obtained by different observers, each using the same method of counting. The individual differences appear to depend upon the microscopic technique and visual acuity of the observer. We are convinced that it is impossible to standardize the platelet count so that the results of even expert technicians will not show significant variation." An approximate minimum error might be expected to lie between 10 and 15 per cent, and including an observer component the error might well be considerably greater. It is therefore rather surprising that previous estimates of error are extraordinarily small; that calculated from Ivanitsky-Vasilenko and Klimova's (1937) results in the Lempert method gives a random error of 0.2 per cent, Dameshek's figures (1932) give an error of 3.8 per cent, and Olef (1935), using an indirect method,

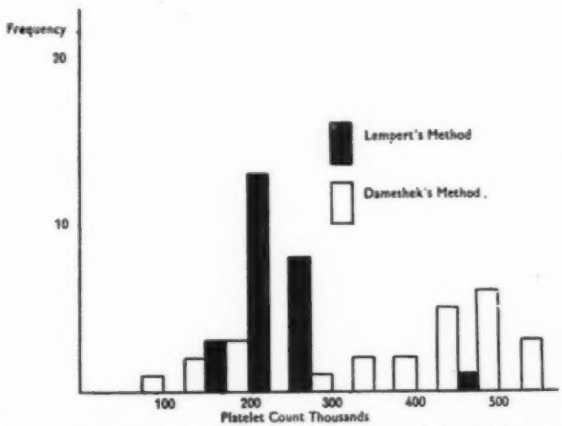

FIG. 8.—Histogram to show the distribution of the individual platelet counts by Dameshek's and Lempert's methods.

gives figures with a coefficient of random variation of 1.8 per cent. It would seem probable that unconscious bias must have been responsible for these very low figures.

In this experiment five observers each made five platelet counts using five pipettes and counting-chamber combinations. The counts were taken at hourly intervals from a different finger of the same subject. Thus the errors due to observers, calibration of pipettes, and counting chambers, and the time of taking the sample could all be calculated.

TABLE VI

THE COMPONENT ERRORS IN COUNTING PLATELETS EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES OBTAINED BY THE TWO METHODS: THE ERRORS FOR PIPETTES AND ORDER OF TAKING THE SAMPLES ARE OMITTED BECAUSE THEY ARE SMALL IN RELATION TO THE RANDOM ERROR

Source of error and mean value	Dameshek's method	Lempert's method
Mean	365,000 c.mm.	246,000 c.mm.
Total coefficient of variation	41%	23%
Error due to observers	33%	13%
Random error ..	22%	17%
Random error (excluding one reading which differed markedly from the majority of observations) ..		6%

The distribution of the individual counts are shown in Fig. 8. Lempert's direct method shows far less variation than does the indirect method of Dameshek, and if one observation which differed markedly from the rest is excluded the random variation approaches that due to the Poisson distribution. It will be seen from Table VI that the superiority of Lempert's method follows a decrease in the difference between the counts of the five observers. In both these methods the errors attributable to the pipettes and time of taking the samples were small in comparison with those of the observers and the random variation.

These results therefore reveal errors well above the probable minimum for the method, an error which could certainly be reduced if one experienced observer made a series of counts. However, for clinical purposes great accuracy is not required and the results obtained by Lempert's method are adequate.

Whole-blood coagulation.—Pauwen and others (1942) studied the coagulation time of normal venous blood. They found that the coagulation time of different normal subjects measured as an average of four tests did not show any very great variation, but that the four tests taken at one time might differ widely. Their figures show a random variation of about 15 per cent.

In this experiment four observations of the whole blood coagulation were made by each of the three methods on one subject on ten consecutive days. In the Lee and White method the order in which the tubes were filled with blood was recorded. In the Dale and Laidlaw method the four observations were made consecutively, on the same finger, a second prick often being necessary. The order of the tests was recorded. In the capillary tubing method all the tubes were filled from the same prick. In this way differences between the methods, between the order of taking the samples, and between samples taken on different days could be estimated.

The different methods are known to give distinct values for the coagulation time. From Fig. 9 it

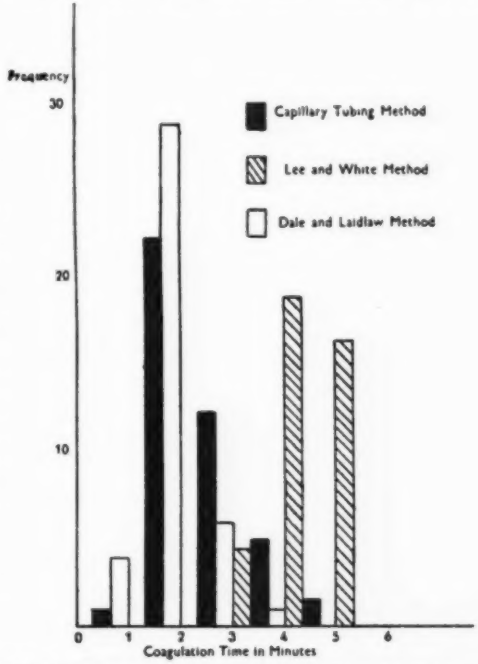

FIG. 9.—Histogram to show the distribution of individual determinations of the whole blood coagulation time by three methods.

will be seen that the individual readings for the Lee and White and the Dale and Laidlaw methods showed approximately the same dispersion about their means, but since the mean value for the Dale and Laidlaw method is lower the error is relatively larger (Table VII). In the capillary tubing method

TABLE VII

THE COMPONENT ERRORS IN THE MEASUREMENT OF BLOOD COAGULATION EXPRESSED AS A PERCENTAGE OF THE MEAN VALUES

Source of error and mean value	Lee and White method	Dale and Laidlaw method	Capillary tubing method
Mean	4.8 min.	1.5 min.	2.0 min.
Total coefficient of variation for tests made over a period of 10 days ..	10.8%	27.5%	29.4%
Total coefficient of variation for tests made on one day	4.7%	15.5%	14.0%
Error due to the order of taking samples on one day	6.2%	20.9%	0
Error due to variations between the different days on which samples were taken	9.7%	22.5%	25.9%
Random error ..	7.0%	25.1%	28.0%

the variation was greater. From an analysis of the constituent errors it was seen that both the methods using capillary blood showed a greater change from day to day than did the method of Lee and White. The Dale and Laidlaw method also showed a big discrepancy between the tests taken at one time, and from Table VIII it will be seen that the first test had a longer coagulation time. From the method of taking the samples this was probably due to a larger amount of tissue juice in tests after the first. In the Lee and White method the first tube often coagulated more quickly than the others, and presumably thromboplastin from the needle was included in this specimen.

These results illustrate the small variability of the coagulation time as tested by the Lee and White method. The technique appears to be more reliable than that of Pauwen and others (1942).

Red cell fragility.—Many of the factors which influence the red cell fragility curve, including the degree of anaemia, oxygenation of the blood, the *p*H of the saline solutions, and the temperature of incubation, have been worked out by Dacie and Vaughan (1938). They used a technique in which all these factors were considered to determine the red cell fragility in fifty normal subjects, and thus set limits to the normal range. Since discrepancies between the results of different observers have arisen in relatively simple methods, it seemed possible that the red cell fragility curve, as used in a routine laboratory, might show a wider range of variation in normal people than was recorded by the originators of the technique.

TABLE VIII

A COMPARISON OF THE MEAN VALUES FOR FOUR CONSECUTIVE ESTIMATIONS OF BLOOD COAGULATION MADE BY THREE DIFFERENT METHODS (THE FIGURES GIVEN ARE THE AVERAGES OF THE VALUES OBTAINED ON TEN DIFFERENT DAYS)

Method	Order of test			
	1	2	3	4
Lee and White	4.4 min.	4.8 min.	5.1 min.	5.0 min.
Dale and Laidlaw	2.04 min.	1.38 min.	1.32 min.	1.40 min.
Capillary tubing	1.92 min.	2.12 min.	2.05 min.	2.10 min.

In this experiment, therefore, five observers measured the red cell fragility in fifty normal subjects. Ten observations were made by each of three observers from one laboratory, and twenty by two observers from a second laboratory. The same saline solutions were used throughout. In most cases duplicate tubes were set up to reduce the errors of pipetting. In addition the red cell fragility of one normal subject was tested on several different days.

The mean value for the median corpuscular fragility, 0.392 per cent, was higher than that of Dacie and Vaughan (1938), 0.366 per cent, because less blood was used in proportion to the volume of saline. The readings of the five observers, considered separately, showed variation in relation to their respective mean values which was comparable to that of Dacie and Vaughan (1938), but, since the observers' mean values were different, the total range was wider (Fig. 10). Thus, if the red cell fragility is always measured by one observer a range of variation comparable to that established by Dacie and Vaughan will include all the readings on normal people. On the other hand, if the test is made by a number of observers wider limits of normality must be set. The diversity of the red cell fragility curve in one normal subject

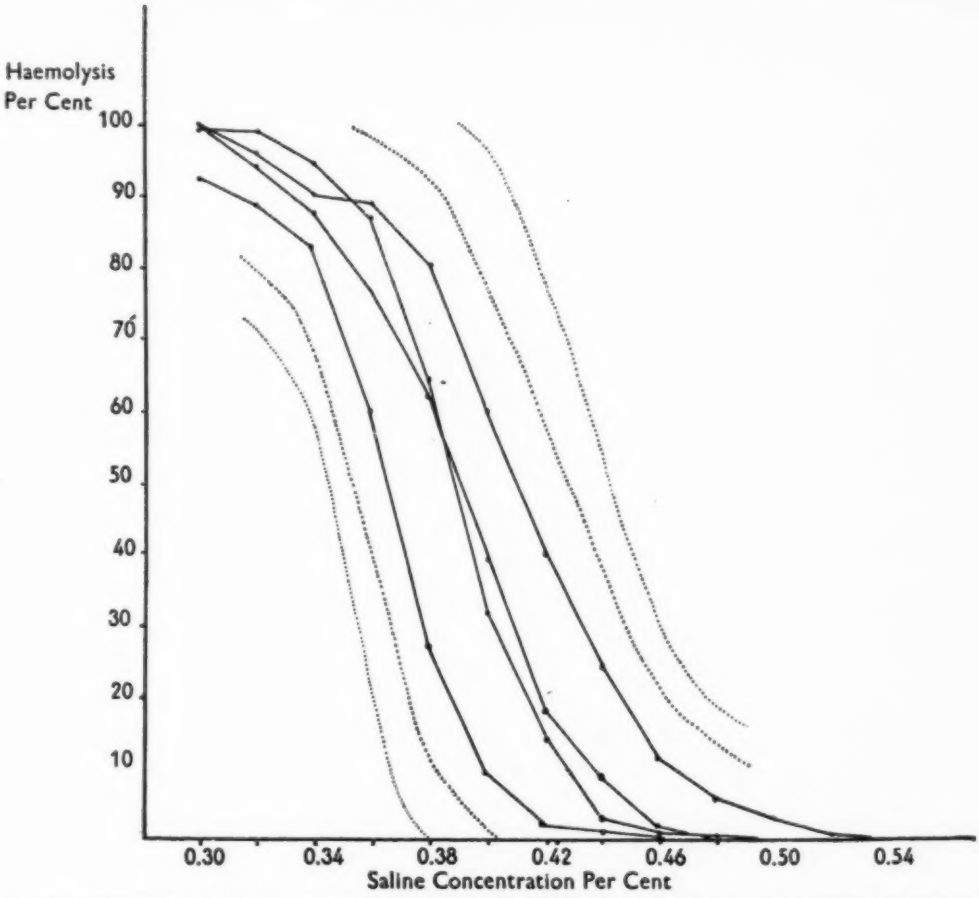

FIG. 10.—Diagram to show the mean values for the red cell fragility curve in normal people obtained by different observers (continuous lines). The limits of probable variation referred to in the text are shown by discontinuous lines.

(Fig. 11) also suggests that it would be unwise to accept a narrow concept of abnormality.

In Fig. 10 the mean values for the four sets of observations are given ; above and below these are indicated two limits of the probable variation in normal people. The narrower range represents one standard deviation above and below the means of the more extreme observers. These limits should include nearly 90 per cent of all the readings. The wider limits represent two standard deviations above and below the extreme observers, and these should include almost all normal fragility curves.

Discussion

In all these investigations emphasis has been placed on the use of a particular method as a routine procedure. We have therefore deliberately not required the observers to have specialized skill in any one technique. It may well be thought that the large errors obtained, in most cases larger than any previously recorded, are proof that none but those highly skilled can carry out these investigations adequately. To some extent this is true, for many of the techniques can be used with far greater precision. The Haldane method for haemoglobin estimation, for example, a notoriously

unreliable method in routine practice, can give very uniform results in the hands of one observer (Macfarlane, 1945). Again, several of the observers taking part in these experiments made reticulocyte counts on one sample of blood which showed very little variation, and Price-Jones (1933) and Mogensen (1938) have demonstrated the high precision of the Price-Jones curve. Though one skilled worker may obtain very uniform results it does not follow that the figures of different skilled observers will agree. In the Haldane haemoglobin estimation, for example, Macfarlane (1945) has shown that the skilled observers almost invariably disagreed; in this experiment the reticulocyte counts of the different observers varied, and a similar observation was made by Aggeler and others (1946) in counting platelets.

From this it is clear that, were it possible for one observer to carry out all the investigations, the variability of the results should approximate to the minimum error of the method. The probable minimum errors of some of the methods discussed

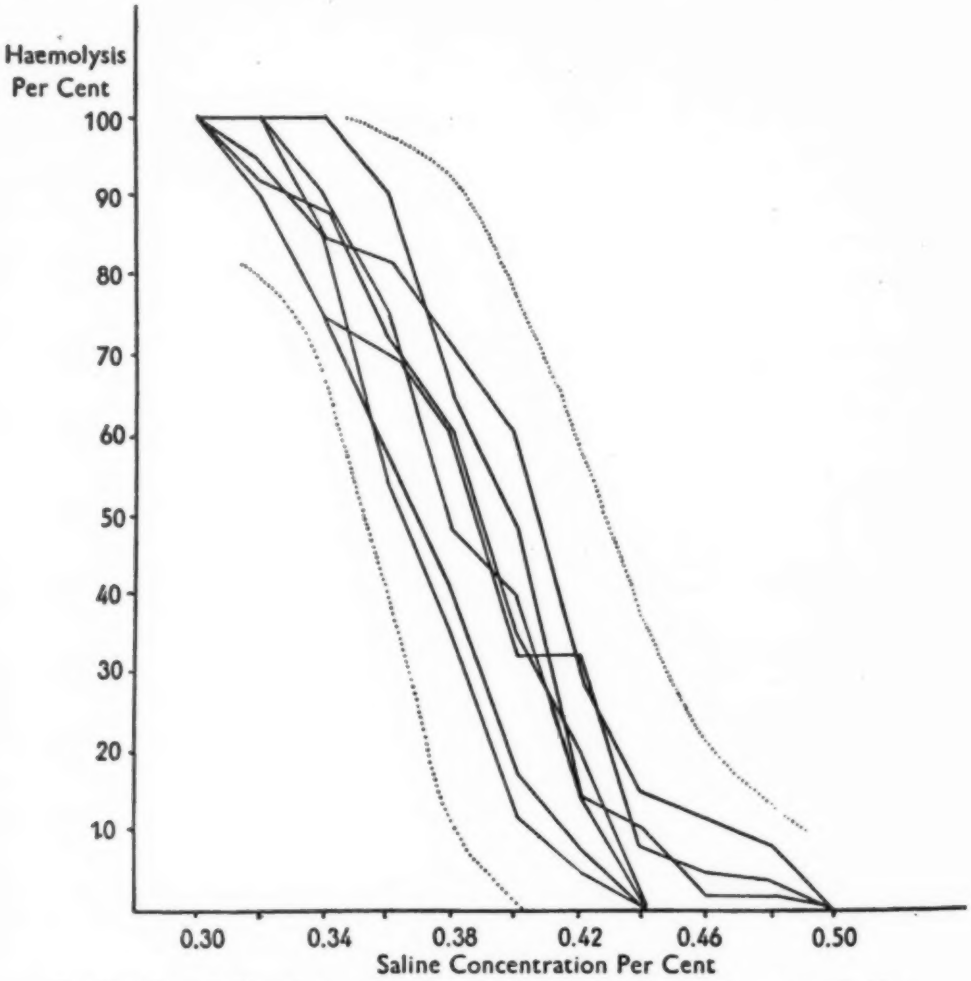

FIG. 11.—Diagram to show the range of variation in the red cell fragility curve in one normal subject.

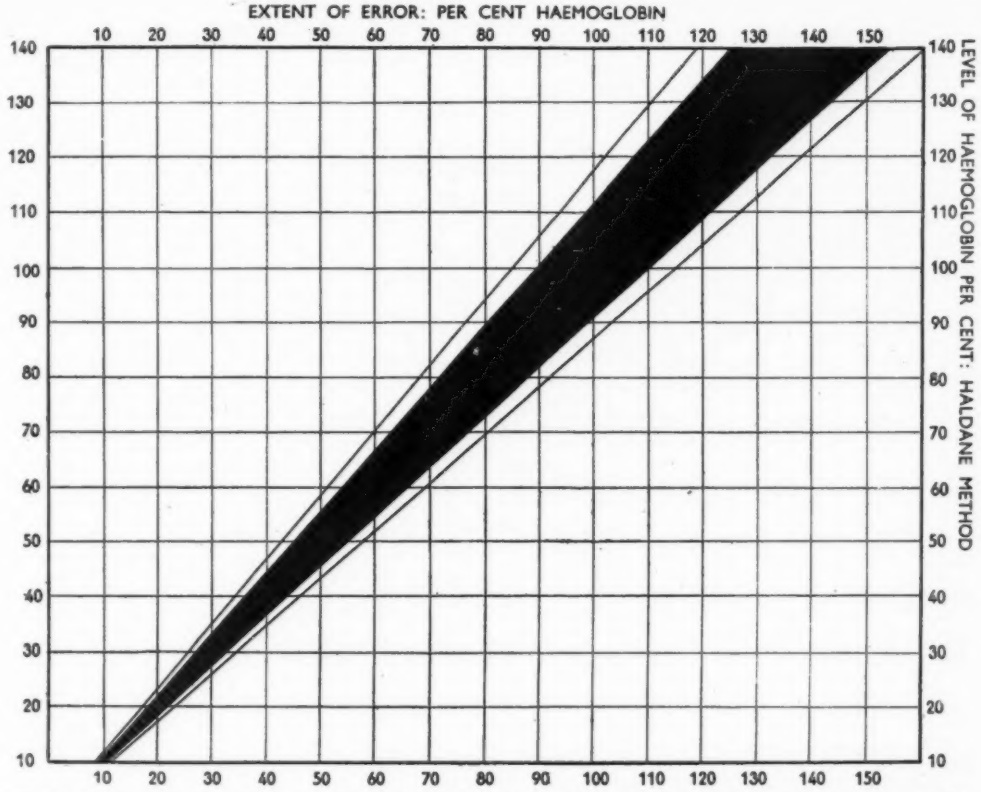

FIG. 12.—Diagram to show the error of haemoglobin determination by the Haldane method at levels of haemoglobin from 10 to 140 per cent. On the vertical axes the magnitude of the error is indicated. The solid area represents the range within which nineteen out of twenty repeated estimations on one sample will fall. The outer lines give the upper and lower limits that might be expected to contain nineteen out of twenty pairs of estimations on the same blood sample. In any two estimations on the same patient, therefore, a difference greater than these limits would have to be obtained before any significant change in haemoglobin could be inferred. The value is referred to as "the significant difference." The values for significant difference in Figs. 12, 13, and 14 are approximations because the error for an increase in the value is slightly different from that of a decrease. (The error should be calculated from $2\sqrt{\quad + S_2^2}$ for any given difference and is in fact calculated from $2\sqrt{s_1^2}$ at the upper level and $2\sqrt{s_2^2}$ at the lower level.)

Example of the Use of Fig. 12: If the haemoglobin is recorded as 70 per cent (vertical axis) repeated observations would be expected to lie between 63 and 76 per cent (horizontal axis). If the haemoglobin is 70 per cent on one occasion, a second observation must exceed 81 per cent or fall below 59 per cent before any significant change can be inferred.

are given in Table I. These limits are important, for it is common for skilled observers to improve on them. In the red cell count, for example, any skilled technician can, as Wintrobe has said, obtain results on one sample of blood that will agree within 200,000 cells. Again some of the recorded figures for platelet counting show the most extraordinary agreement (Olef, 1935; Ivanitsky-Vasilenko and Kilmova, 1937). This uniformity, which would be impossible for a mechanically impartial recorder, is the result of an unconscious bias which was clearly demonstrated by Macfarlane (1945) in the case of the Haldane haemoglobin determinations. Macfarlane showed that observers could use this method with a greater uniformity of results when provided with

graduated tubes than when only plain tubes were supplied. In the case of the red cell count this excessive accuracy can be shown to decrease, rather than increase the reliability of the results (Biggs and MacMillan, 1948).

The question of importance is the course which should be followed in a routine laboratory. Investigations of which fifty to a hundred may be made in one day cannot all be made by one highly skilled observer. With these, it is essential to select those methods which give reasonably good results in the hands of the "average" observer. The methods of most importance are those which assess the degree of anaemia and the response to treatment. The selection of the method for haemoglobin estimation is clearly important and, as Macfarlane and others (1948) have shown, the methods with an error approaching the minimum are the neutral grey photometer using oxyhaemoglobin, the Sahli-Zeiss comparator using acid haematin, and the Duboscq photometer using various pigments. In addition, the haematocrit gives very reliable results and from these two observations the mean corpuscular haemoglobin concentration may be calculated. These measurements, together with the pathologist's opinion on the appearance of the blood film, give the best information available for routine diagnosis except in the macrocytic anaemias, where some assessment of cell size is necessary.

The mean corpuscular volume and colour index are at present routine procedures in all haematological laboratories, where their vagaries give rise to constant irritation. The labour involved by these measurements is, in our opinion, not justified by the results which they give. There are few pathologists who would accept the figures recorded by either of these indices if they were not borne

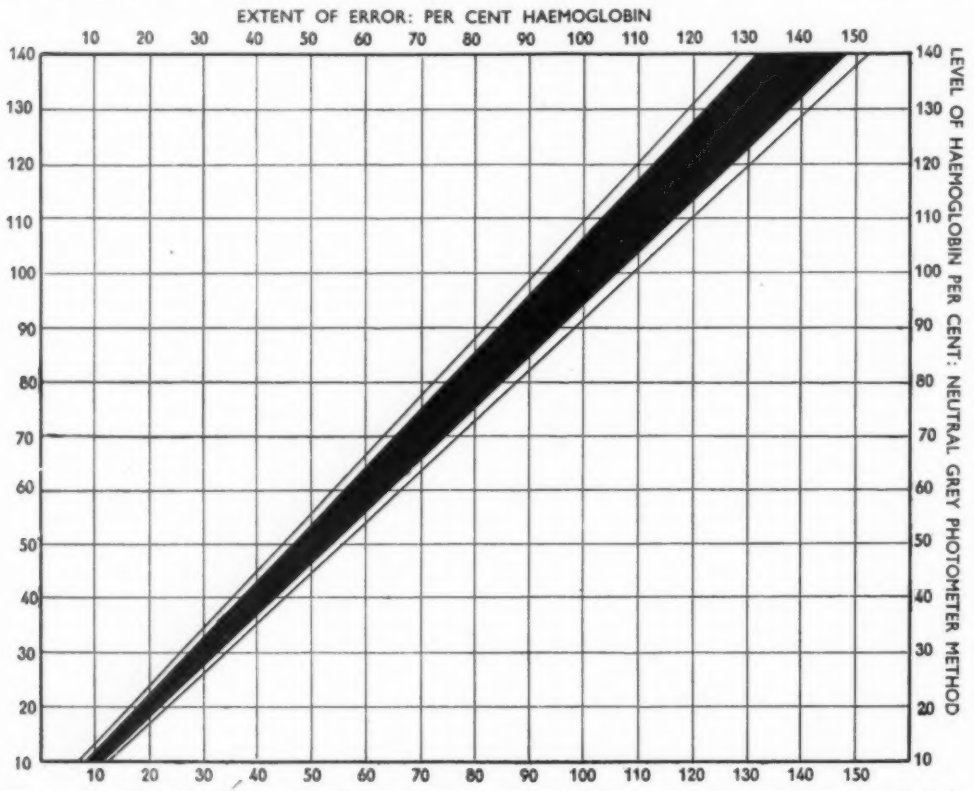
EXTENT OF ERROR: PER CENT HAEMOGLOBIN
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
140 130 120 110 100 90 80 70 60 50 40 30 20 10
LEVEL OF HAEMOGLOBIN PER CENT: NEUTRAL GREY PHOTOMETER METHOD

FIG. 13.—Diagram to show the error of haemoglobin determination by the neutral grey photometer method for levels of haemoglobin from 10 to 140 per cent (see legend to Fig. 12).

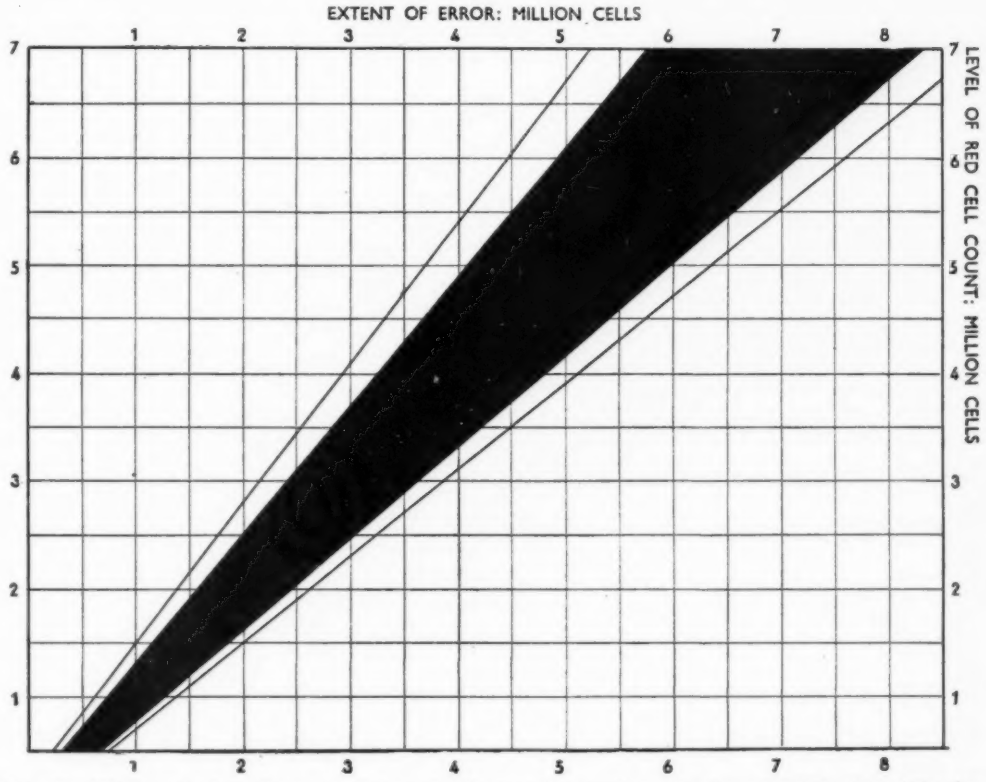

FIG. 14.—Diagram to show the error of the red cell count for counts varying from 50,000 to 7,000,000 using a 1/200 dilution of blood, and counting of eighty small squares in a Neubauer counting chamber (see legend to Fig. 12).

out by the appearance of the blood film. This fact in itself illustrates a conscious or unconscious conviction that the figures are not reliable, a conviction well supported by these investigations. The only exception which must be made is in the case of macrocytic anaemia in laboratories where the drawing of a Price-Jones curve is difficult. In these cases repeated observations will give a broad general impression of the trend of cell size.

In the case of the reticulocyte count, where despite all care different skilled observers tend to disagree, the simple procedure of encouraging one observer to make all the counts on any one patient will increase the reliability of that patient's record.

In the case of investigations less frequently carried out, two possibilities exist. Single observers may be trained to attain specialized skill until their errors approach the minimum for the method. Alternatively, the techniques may be carried out by a number of competent observers, when the range of their errors will be comparable to those recorded here. If the special investigation is complicated and the results require to be accurate, as for example in prothrombin determinations, the former procedure is preferable. On the other hand, many of the investigations need not be measured with very great precision for ordinary clinical purposes, and the results of the "average observer" are probably adequate. It would seem to us preferable to have a laboratory staffed by workers competent to carry out any investigation that could reasonably be requested, than a staff of specialists in single techniques.

The final problem is the application of these concepts of error to routine practice. The errors that we have calculated in most cases apply only

to the readings recorded. The error of haemoglobin determination by the Haldane method, for example, is relative to the level of haemoglobin. Thus an absolute error of 5.2 per cent at 100 per cent haemoglobin is reduced to 2.6 per cent at 50 per cent. The alteration in error with change in haemoglobin level is shown in Fig. 12. This diagram shows the range within which repeated observations on one sample may be expected to fall (solid area), and the difference between two haemoglobin readings which may be considered significant. In this method doubling the volume of blood used for low concentrations of haemoglobin would be expected to give little increased accuracy and must introduce additional error from the filling of pipettes on two occasions.

In the neutral grey photometer method the error of reading the photometer remains constant and though this error is small it becomes relatively more important at low levels of haemoglobin. Thus a slight reduction in error will result from doubling the amount of blood when the haemoglobin reading is less than 30 per cent. The errors of this method are shown in Fig. 13.

In the red cell count, as Berkson and others (1940) have shown, the error tends to increase with a decrease in the number of cells counted. Fig. 14 shows the errors that will occur with a constant technique of counting eighty small squares in a dilution of 1/200 using a Neubauer counting chamber. Some increase in precision will follow an increase in the number of cells counted. Using the technique outlined above, a count of one million cells will have an error of 12.6 per cent. By using a dilution of 1/100 and counting 160 small squares the error may be reduced to 9.5 per cent. Further reduction in error can be obtained by using two pipettes and two counting chambers. In the example given above the error would in this way be reduced to 7.8 per cent if a total of four hundred cells were counted (eighty small squares from each counting chamber using a dilution of 1/100).

The random error of the haematocrit is probably independent of the level of packed cells and thus will be proportionately greater at low haemotocrit readings. However, this error is sufficiently small to be negligible in comparison with the errors of other methods in use.

When reticulocyte counts are made by many different observers, the errors are so large that no useful limits of probable variation can be set. In the hands of one reasonably skilled observer the error approaches that of the binominal distribution. The limits of error for counts of five hundred and a thousand cells are shown in Table IX.

TABLE IX

THE ERROR IN ESTIMATING THE PERCENTAGE OF RETICULOCYTES WHEN ONE COMPETENT OBSERVER COUNTS 500 OR 1,000 RED CELLS WITH DIFFERENT PROPORTIONS OF RETICULOCYTES

Percentage of reticulocytes	Range within which the percentage of reticulocytes should fall is 19/20 repeated counts on one sample		Significant difference in percentage of reticulocytes	
	500 cells*	1000 cells†	500 cells*	1000 cells*
1	0–2	0–2	1.5	1.5
2	1–3	1–3	2.0	1.5
3	1–5	2–4	2.0	1.5
4	2–6	3–5	2.5	2.0
5	3–7	4–7	3.0	2.0
6	4–8	5–8	3.0	2.0
7	5–9	6–9	3.0	2.5
8	6–10	6–10	3.5	2.5
9	6–12	7–11	3.5	2.5
10	7–13	8–12	4.0	2.5
15	12–18	13–17	4.0	3.0
20	17–23	18–23	5.0	3.5
25	21–29	22–28	5.5	4.0
30	26–34	27–33	6.0	4.0
35	31–39	32–38	6.0	4.5
40	36–44	37–43	6.0	4.5
45	41–49	42–48	6.5	4.5
50	46–54	47–53	6.5	4.5

* Calculated from the standard deviation.
† From Snedecor (1946).

In platelet counting the error is so large that only conspicuous and progressive changes should be considered significant.

Summary

1. A series of experiments was devised to assess the errors inherent in the routine use of some common haematological methods. The results are summarized in Table I and in the figures illustrating the text.

2. Some of the sources of error were disclosed, and among these disagreement between observers was important.

3. An increase in the reliability of routine work will follow the selection of those methods which give the best results in the hands of the average laboratory worker.

4. In the diagnosis of anaemia the most precise measurements found in this investigation were: haemoglobin determination using oxyhaemoglobin and a neutral grey photometer, the haematocrit, the mean corpuscular haemoglobin concentration, and the mean cell diameter derived from the Price-Jones curve.

5. The red cell count has an error of 9 per cent and thus the colour index and mean corpuscular volume are too variable to record the small differences of interest to the clinician. The routine use of these indices might be restricted to the study of macrocytic anaemia in laboratories where a Price-Jones apparatus is not available.

6. The most important error in counting reticulocytes arises from disagreement between observers; this will be minimized if the same laboratory worker makes all the counts on any one patient.

7. The platelet count by both Dameshek's and Lempert's methods showed a large standard deviation, but Lempert's method was preferable.

8. Five observers recorded different mean values for the red cell fragility in normal people. In the routine laboratory it would be wise to use a wider concept of normality than that established by Dacie and Vaughan (1938).

We thank the many doctors and laboratory technicians who willingly took part in these experiments. The statistical analysis of the Price-Jones curve and the red cell fragility curve were made by Mr. Finney, whom we also thank for assistance and advice in all the mathematical difficulties that arose. We should like to thank Dr. R. G. Macfarlane for his continued interest and help throughout the investigation.

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THE ERROR OF THE RED CELL COUNT

BY

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The staffs of haematological laboratories spend much of their time counting red cells. The precision of this estimation is therefore of practical importance, and many experiments have been designed to establish the error of the technique. In spite of this, the most widely divergent views are still held as to the reliability of single observations.

In 1881 Lyon and Thoma showed that the standard error of counts made on the same sample of blood was roughly proportional to the square root of the number of cells counted. Thus in a count of 100 cells the standard error would be roughly $\sqrt{100}$, and repeat counts on the same sample might by chance vary from 80 to 120 cells, or, in counts of 500 cells, the standard error would be approximately $\sqrt{500}$ or 4.5 per cent. In 1906–7 " Student " confirmed and stated more exactly the findings of Lyon and Thoma when he showed that the scatter of cells in a haemocytometer followed the Poisson distribution, in which the standard error is equal to the square root of the mean for the distribution. Thus, if 500 counts are made on one sample of blood and the mean value for each is 100 cells, the standard error is $\sqrt{100}$ and the majority of counts would show a range from 80 to 120 cells. From these observations it appears that there is an irreducible error in making a red-cell count which is approximately proportional to the square root of the number of cells counted.

In 1935 and 1940 Berkson, Magath, and Hurn again demonstrated the Poisson distribution of the red cells in a haemocytometer and made a thorough analysis of the other errors inherent in this technique. They showed that in a normal count the standard error is 7.7 per cent. An error of this magnitude implies that two counts on a normal sample of blood may differ by up to 1,100,000 ($\pm \sqrt{2S^2}$). In 1947 Hynes showed that the distribution of cells varies from one end of the coverslip to the other, and unless there is a reasonable length of coverslip on either side of the ruled area the error may be larger.

Wintrobe (1946) on the other hand maintained that repeat counts on a single sample should agree to within 200,000 cells, implying a standard error of 1.4 per cent, and several workers have published results in which this error has been achieved or even bettered (Mayers, 1922; Smith, 1931; Wintrobe, 1934; Price-Jones and others, 1935). This error is considerably less than that due to the Poisson distribution alone. Wintrobe (1946) does not recognize this distribution because he holds that the number of cells enumerated in the five separate squares of a red-cell count should not differ by more than 18. A greater internal variation is thought to indicate incomplete mixing of the contents of the pipette, and according to this criterion counts showing a greater variability should be rejected. In the Poisson distribution, on the other hand, differences of up to 40 cells might occur by chance. Wintrobe's opinion is apparently shared by the many skilled workers who habitually apply this criterion of adequate mixing. In fact, it is probably true that in spite of Berkson's careful experiments and statistical analysis many believe that Wintrobe's assessment of the error is more nearly true.

Experiments

We have therefore made several experiments in an attempt to find the origin of this apparently irreconcilable discrepancy.

Experiment 1.—Two reliable and skilled technicians made ten counts on a single sample of normal blood, using five pipettes and five counting chambers. In all these experiments one pipette was always used with the same counting chamber. The cells in a volume of 1/10,000 c.mm. of blood in five large squares were counted. The values for each square were recorded

TABLE I

THE NUMBERS OF RED CELLS RECORDED FOR EACH OF THE COUNTS IN EXPERIMENT 1

Observer	Pipette									
	1		2		3		4		5	
1	503	481	467	498	496	496	494	497	496	492
2	481	481	489	482	482	477	480	478	465	458

separately and the workers were requested to apply Wintrobe's criterion for complete dispersion of the cells in the pipette; that is, to reject counts showing a variation greater than 18 cells between the five squares. They were aware that they were dealing with only one sample of blood, and were encouraged to compare results. The figures obtained showed close agreement (Table I). The first technician's counts had a coefficient of variation of 1.9 per cent and those of the second 2.1 per cent. In this experiment the technicians separately obtained a precision closely approaching Wintrobe's requirements. There was, however, a fairly large difference between the two sets of results, one giving a mean value of 477 and the other of 492.

TABLE II

THE NUMBERS OF RED CELLS RECORDED FOR EACH OF THE COUNTS IN EXPERIMENT 2

Observer	Pipette				
	1	2	3	4	5
1	430	404	437	453	517
2	475	405	455	412	443
3	437	440	508	435	488
4	488	449	485	463	505
5	458	397	458	436	447

Experiment 2.—Five technicians made five counts on one sample of normal blood using five pipettes and five counting-chambers. The blood was divided into five fractions, which the technicians had no reason to believe were derived from the same person. They did not compare results, but were requested to use Wintrobe's criterion for complete mixture of the contents of the pipettes. From Table II it will be seen that these counts show far greater variation than those of the first experiment. The coefficient of variation is now 7.6 per cent.

Experiment 3.—Five doctors who, though competent in the technique, were not engaged in red-cell counting as part of their daily work, made ten counts on the sample of blood used in Experiment 2, using ten pipettes and counting-chambers. These workers inevitably made their counts more slowly than the technicians, and—choosing the squares at random—were unable to obtain a close agreement between the counts for the different squares; a difference of up to 40 cells was allowed. The results show an even greater variation, and the standard error is 9.5 per cent (Table III).*

* That this error is greater than that recorded by Berkson may probably be due to two main factors: (1) Berkson used an electric counter and enumerated the cells from photographs, whereas in these experiments the cells were counted by eye. (2) Berkson's experiments were made by one observer, and our experiments show that an error of 3 per cent may be attributed to differences between observers.

TABLE III

THE NUMBERS OF RED CELLS RECORDED IN EACH OF THE COUNTS IN EXPERIMENT 3

Observer	Pipette									
	1	2	3	4	5	6	7	8	9	10
1	427	372	418	440	349	484	430	416	449	464
2	434	420	385	472	415	420	415	396	439	424
3	480	421	473	496	474	411	472	423	502	488
4	451	369	500	464	444	410	422	396	459	471
5	462	453	450	520	489	409	508	347	440	391

Discussion

These experiments show that the magnitude of the error depends entirely on the condition of the experiment. The main components of error from an analysis of variance are shown in Table IV.

TABLE IV

THE COMPONENTS OF ERROR DERIVED FROM AN ANALYSIS OF VARIANCE IN EXPERIMENTS 1, 2, AND 3

Experiment	Total error %		Error due to observers %	Error due to pipettes and counting chambers %	Random error %
	Separate observers	Both observers			
I	1.9 2.1	3.6	2.0	0	2.5
II	7.6		2.9	4.8	5.1
III	9.5		3.1	4.2	7.8

The striking feature is the progressive increase in random error. In experiment 1, which is similar to those described by Wintrobe (1934) and Price-Jones and others (1935), the error is small if the results obtained by the two technicians are considered separately. The random error is well below that of the Poisson distribution (4.5 per cent). There is no component of error referable to the calibration and filling of pipettes and calibration of counting chambers, factors well recognized as a source of error in haematological technique. Moreover, it can be shown statistically that there is less than a 1/100 probability of obtaining mean values as widely divergent as 477 and 492 from the same sample of blood when the counts for each observer are so uniform.

Since there are good reasons for suspecting that the standard error recorded in the first experiment is not a true estimate, and since workers not engaged in red-cell counting as a routine are unable to obtain any close agreement, it is perhaps of interest to examine the process of training which leads to the making of uniform counts. This has been described by Emerson (1921), who recommended his medical students to make repeated counts on one person's blood until they achieved an agreement of 200,000 cells. He says, "Some students attain this accuracy quickly. Some, however, repeat this daily counting for twenty or thirty or even more days before their work is satisfactory to themselves or to us. By this time they have certainly learned wherein lies the error of their technique. It is of interest that the most careful ones sometimes make the greatest errors since they take too much time where speed is essential." It seems probable that a training of this sort leads to an unconscious bias in favour of agreement between counts, a bias that was well demonstrated in Experiment 1.

In Experiment 2 there was no reason to obtain any agreement between the five sub-samples of blood, but the values for the last four squares of each count were biased by that of the first square (because an agreement between the five squares of 18 cells or less was required). In the third experiment the full range of random variation was shown.

It seems probable that any artificial reduction in the true variability will lead to a decrease rather than an increase in precision; nevertheless it is possible that a skilled observer trained to make uniform counts on a single sample of blood might be able to select from each counting chamber squares typical of the whole field, which would estimate the mean value more precisely than a random selection. Experience in many sampling problems, however, suggests that subjective selection of this kind is a frequent source of unconscious bias. To test this point a further experiment was made.

Experiment 4.—Five technicians made routine red cell counts on eighteen different samples of blood and recorded separately the figures for each square. The counting chambers were then taken and the cells in all the squares in the ruled area were counted. The results of one such experiment are shown in Table V. The mean value per square obtained as an average for the whole field is clearly a better estimate of the true mean value than that derived from any five

TABLE V

ONE OF THE 18 COUNTS MADE IN EXPERIMENT 4.

(In the top row are the separate counts for each of the five squares obtained by the technicians. The counts for the separate squares of the whole field are recorded below.)

Technician's counts	80	82	79	82	77
Counts for whole field	77	69	62	69	70
	64	94	77	71	67
	85	73	66	67	72
	62	89	65	72	77
	64	64	67	77	62

squares. Nevertheless if the five squares were chosen at random their mean value should not differ significantly from that for all the squares. When the mean values for each selection of five squares obtained by the technicians are compared with the corresponding mean for the whole field, several of the eighteen samples show a large difference, and statistical tests show that the dispersion about the field means is significantly greater than would be expected for the means of random samples. In other words, on an average, a random selection of five squares would give a more trustworthy estimate of the field mean than would the set of five selected by one of these technicians; the insistence on agreement between counts for the five selected squares has achieved a bias in estimation rather than an increase in precision.

Conclusions

It is concluded that: (1) the training of workers who make routine red cell counts should be a training in accurate counting rather than a training in achieving agreement between replicate counts; (2) in making a red cell count a difference of up to 40 cells should be allowed between the five separate squares counted; (3) from the most reliable estimates the standard error of the red-cell count lies between 8 and 10 per cent.

We should like to thank Dr. R. G. Macfarlane for his continued interest and advice, Mr. Finney for assistance with the statistical analysis, and graduate assistants and technicians of the department of haematology for their patience and willing co-operation in carrying out the experiments.

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A FURTHER CASE OF ANTI-LUTHERAN IMMUNIZATION, WITH SOME STUDIES ON ITS CAPACITY FOR HUMAN SENSITIZATION

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The "Lutheran" antibody was first described by Callender, Race, and Paykoç (1945) in the serum of a patient who developed a number of antibodies in response to multiple transfusions of blood. Callender and Race (1946) showed that the red cells of 8 to 9 per cent of the English population were agglutinated by this antibody, that it was inherited as a Mendelian dominant, and that it was unrelated to the ABO, MN, P, or Rh blood groups.

This paper reports a second example of this antibody found during the investigation of a transfusion reaction, and some attempt is made to assess the immunizing capacity of the Lutheran antigen.

Case History

In February, 1946, a man (A.T.) aged 38 with cirrhosis of the liver was admitted to the Radcliffe Infirmary (Reg. No. 10771/46) for treatment of bleeding oesophageal varices. He had suffered from recurrent haematemeses since 1931, and had been transfused, latterly at about yearly intervals. In the two months before his admission to this hospital he had received four transfusions, most of which had been accompanied by rigors. On March 1, 1946, he was transfused with four pints of group O Rh-positive blood. A slight reaction was noted though not reported till later. On March 7 he received a further two pints of O Rh-positive blood; this transfusion was followed by marked jaundice and haemoglobinuria, and on investigation two days later his blood group was found to be O Rh-negative, with no evidence of surviving Rh-positive cells, thus showing that he had destroyed all the blood transfused. His serum contained an incomplete anti-D antibody (titre 1:128 in concentrated albumin and 1:512 Coombs' titration), the obvious cause of his transfusion reaction. In addition there was an agglutinating antibody active at 20° and 37° C. This was also present at the same titre of 1:16 in the pre-transfusion sample taken on March 1, 1946, which suggests that it had not caused the transfusion reaction. In fact all the bottles given had been cross-matched and found to be compatible and were later shown to be Lutheran-negative. As the serum also agglutinated the cells of a known Lutheran-positive donor, it was investigated in parallel with the original anti-Lutheran serum by Dr. S. T. Callender and Dr. R. R. Race. It agglutinated all of twelve Lutheran-positive and failed to agglutinate all of forty-seven Lutheran-negative bloods. The probability of such a coincidence being due to chance is infinitely small.

During April and May, 1946, the patient was injected with small amounts of Lutheran-positive cells. His subsequent transfusions were compatible both for the Rh and Lutheran factors. His antibody titres in June, 1946, were 1:512 incomplete anti-D (in albumin) and 1:16 anti-Lutheran. A year later the titre of the incomplete anti-D antibody had dropped to 1:64 and the anti-Lutheran antibody reacted weakly at a titre of 1:1. In January, 1948, no anti-Lutheran antibody was detectable in his serum.

The type of agglutination was similar to that previously described—compact agglutinates among many unagglutinated cells—and though with many of the test cells the serum gave slightly stronger reactions at 37° C., other cells were agglutinated more strongly at 20° C.

Serological Investigations

Three hundred and sixteen random bloods were tested with the serum; twenty-six were positive (8.23 per cent). It was possible to distinguish strong and weakly reacting types of positive cells; of the twenty-six Lutheran-positive cells fifteen were strong reactors or L_1 and eleven weak or L_2. When the serum (titre 1 : 16) was absorbed with an equal volume of packed cells the L_1 cells removed all the agglutinin, whereas when it was absorbed with the L_2 cells, which removed all antibody capable of reacting with themselves, it

TABLE

Name and Reg. No.	Recipients			Donors' Rh and Lutheran Group		Serum tested (days)	Immune investigations
	Age	Diagnosis	Rh Group				
E.W. 21754/44	46	Duodenal ulcer	R_0	R_1R_2 R_1r	L + L +	15 25 31	1:16 Anti-Lutheran 1:4 „ „ :2 „ „ — All previous donors L —
A.P. 36929/44	46	Cholecystectomy	R_2	R_1R_2 R_1R_2	L + L +	28 36 72	1:4 „ „ 1:2 „ „ Negative — Previous donors not available
A.C. 45572/45	56	Anastomotic ulcer	R_1	R_1r R_0r	L + L +	11 17	Negative Negative — Previous donors not available
C.B. 57191/46	12	Traumatic splenectomy	N.T.	R_1	L +	13	Negative
A.T. 54323/46	67	Partial gastrectomy	R_1r	R_1r $R''r$ R_1R_1	L + L + L +	30 68	Negative Negative
W.M. 50740/46	59	Prostatectomy	R_2	rr	L +	20	Negative
P.M. 55258/46	32	Hysterectomy and salpingo-oophorectomy	rr	R_1r R_1R_1	L + L +	24 58 98	Negative Negative Negative
H. P.P.		Prostatectomy	N.T.	R_1r N.T.	L + L —	10 42	Negative Negative

N.T. = Not tested.

still agglutinated the more strongly positive bloods. This is analogous to the subdivision of the blood group A into A_1 and A_2 with the α_1 serum.

	Cells		
	L_1	L_2	L negative
Serum before absorption	++	+	–
Serum-absorbed L_1 cells	–	–	–
Serum-absorbed L_2 cells	+	–	–

This result cannot be explained by the dosage effect of homozygous and heterozygous bloods, as the expected frequency of Lutheran-positive homozygotes in 316 bloods would be less than one, whereas fifteen were observed. Five families have been studied, two of the L_1 and three of the L_2 type. In all cases the antigen was present in the same form in all members of the family. The evidence is in favour of there being three allelomorphs of the gene, their approximate gene frequencies calculated from the 316 bloods being L_1 2.5 per cent, L_2 1.8 per cent, and l (Lutheran-negative) 95.7 per cent.

The occurrence of the same type of immune antibody in a second patient in the same hospital within two years suggests that the Lutheran factor is moderately antigenic in man, though this could not be accurately assessed, as both patients had developed other immune antibodies. The first case reported (Callender and others, 1945) was known to be hypersensitive to all blood antigens. It was therefore decided to transfuse Lutheran-negative patients with Lutheran-positive blood to determine the importance of the antigen in transfusion practice. Eight suitable patients were selected from those requiring transfusions, and their sera were examined for atypical antibodies. Each received without any reaction one or two pints of Lutheran-positive blood. Their sera were

re-examined at approximately fortnightly intervals for two months. Two out of the eight patients developed "Lutheran" antibodies of the agglutinating type, but no other antibodies, either agglutinating or incomplete, were detected in any of the eight patients, either on testing against a random panel of test cells or against the blood donors, who were recalled and whose cells were tested against the sera from the patients to whom their blood had been given. Both the patients who developed anti-Lutheran antibodies and one of those who did not respond had received earlier transfusions. In one of these (E. W.) all four donors of the earlier transfusions were tested and found to be Lutheran-negative; in the other two cases the donors were not traced. In both these deliberately immunized patients the anti-Lutheran antibody was detectable in the serum for a short time only. In the case of A. P. it was present at a titre of 1 : 4 twenty-eight days after transfusion, and it fell rapidly and was no longer evident forty-four days later. In E. W. the maximum titre of 1 : 16 was found fifteen days after transfusion; it fell to 1 : 2 during the succeeding fortnight.

Discussion

The chance finding of a further anti-Lutheran antibody and the immunization of two out of eight patients by transfusion with Lutheran-positive blood suggests that the Lutheran antigen is moderately antigenic in man and that supplies of suitable test serum could be obtained fairly readily. However, its antigenic capacity cannot be compared with most of the other blood factors antigenic in man, since very few systematic studies have been made. It had generally been considered that only 2 to 4 per cent of Rh-negative people were sensitized by transfusion of Rh-positive blood, but Hattersley (1947) has shown that eleven (55 per cent) of twenty Rh-negative Servicemen who had been previously transfused developed anti-Rh agglutinins. Diamond (1948) has confirmed this high incidence of immunization of Rh-negative subjects transfused with blood from unselected donors. In his series of over 500 cases, 46 per cent contained anti-Rh agglutinins. Other specific antibodies developing in response to transfusion have occasionally been reported. It is probable that they occur more readily than is generally recognized, as they are usually detected only in the investigations of a transfusion reaction or in the cross-matching of blood for a further transfusion.

Because of the rarity of the Lutheran antigen, its role as a potential cause of transfusion reactions is remote when unselected blood is used. In contrast to the anti-Rh agglutinin the serum titre of the anti-Lutheran antibody does not seem to be well maintained. In the case reported by Callender and Race (1946) the antibody was demonstrated in the serum for only about seven weeks, and though it was present in our first case for sixteen months after the first observation the titre had fallen from 1 : 16 to 1 : 4 in five weeks, subsequent reimmunization probably contributing to its persistence. In the experimentally immunized patients the titre was falling rapidly during the second month after transfusion. There was no indication of clinical incompatibility of transfusion in either of these recipients, though the donor cells must have been rapidly eliminated in case E. W., where an antibody titre of 1 : 16 was found fourteen days after transfusion; and though some changes in the peripheral haemoglobin levels were observed during this time these were not significant, as the patient was losing blood from a bleeding peptic ulcer.

Although the Lutheran antigen has not yet been proved to be the cause of a clinical transfusion incompatibility, it should, in view of its potential immunizing capacity, be considered when a transfusion reaction is being investigated.

Summary

A second example of human immunization to the Lutheran factor is described. In eight Lutheran-negative patients purposely transfused with Lutheran-positive blood, two developed anti-Lutheran antibodies. In none of the cases as yet described was there evidence of clinical incompatibility.

It has been found that there are strongly and weakly reacting Lutheran-positive cells, L_1 and L_2, analogous to the A_1 and A_2 groups. The importance of the Lutheran factor as a blood antigen is discussed.

We thank Dr. R. R. Race and Dr. S. T. Callender for their confirmation of the specificity of the first serum encountered, and Dr. J. Grant for her co-ordination of the selected transfusions.

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SOME OBSERVATIONS ON THIOURACIL NEUTROPENIA, WITH SPECIAL REFERENCE TO THE STERNAL MARROW

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It is generally agreed that leucopenia, with its clinical sequelae, is the most important and occasionally serious toxic manifestation in treatment with the widely used thiourea group of drugs (Lesses and Gargill, 1945; Morgans, 1947; Dunlop, 1947; etc.). The average incidence of agranulocytosis in patients so treated appears to be approximately 2 per cent (Moore, 1946; Van Winkle and others, 1946), and 28 per cent of sixty-one cases of this condition reviewed by Morton (1947) died. In view of these and similar findings, it is considered that the results of an investigation of the sternal marrow in cases of thiouracil neutropenia may be of some interest.

A series of six patients who developed neutropenia during the year 1947, while under treatment with methyl (4) or propyl (2) thiouracil, will be considered. The criterion of neutropenia was taken as a peripheral absolute granulocyte count of less than 1,500 per c.mm. of blood (Osgood and others, 1939). A differential count of 500 nucleated marrow cells was made in each case, except in No. 1, in which 1,000 such cells were counted. In none was there evidence of a primary blood dyscrasia which might be associated with an absolute neutropenia. Brief clinical details of these patients are given in the appendix to this report (p. 299).

The Six Cases

Case 1.—The sternal marrow was first examined on April 3, 1947, when only 64 granulocytes per c.mm. were found in the peripheral blood. Stained films showed normal numbers of earlier, with very scanty later, myeloid forms (see Table, Case 1*a*). There appeared to be a rise in lymphocytes and plasma cells, and a normoblastic reaction (she had hypochromic anaemia; Hb, 70 per cent Hellige—100 per cent = 14 g. per 100 ml. blood—on April 3, 1947). Morphological abnormalities were seen in 55 cells of the myeloid series. Thus some myeloblasts showed, in varying combination, an irregularly shaped nucleus, and scanty, irregularly staining, vacuolated cytoplasm, with irregular outer margin and pseudopodium-like formation (Plate I, Figs. 1 and 2). Promyelocytes with an abnormally large, often irregular nucleus, and cytoplasm showing the features mentioned above, along with coarse granulation (Figs. 3–7), could be seen. The large numbers of cells reported as bare nuclei throughout the series showed, in the main, the general morphology of myeloblastic or premyelocytic nuclei (Fig. 8). Some were degenerated mature granulocytes (Fig. 9).

The cytoplasm of the neutrophil myelocytes showed the abnormal features observed in the premyelocytes, except that the granules were often ill-defined, and seemed to fuse with the cytoplasm (Fig. 10). Irregularity of nuclear outline could be seen in one cell.

The single metamyelocyte encountered also showed irregularly staining cytoplasm with ill-defined granulation, while the neutrophil polymorph exhibited similar granulation, along with an abnormally pale, vacuolated cytoplasm and a pyknotic nucleus.

The sternum was punctured for the second time on April 5, 1947. No granulocytes were then seen in the peripheral blood smears. Examination of the marrow (Table, 1*b*) showed even more marked myeloid suppression than on April 3, the promyelocyte level being especially affected. In general, the myeloid morphology resembled that seen in the first marrow specimen. Additional features worthy of note amongst the 56 abnormal cells were: one myeloblast showed a partly segmented nucleus, the lobes being joined by a relatively narrow chromatin band (Fig. 11); and the promyelocytes showed the ill-defined granules previously mentioned (Figs. 8, 12, and 13). Some neutrophil myelocytes were abnormally large, and granules were often absent from the cytoplasm, or were of very coarse type (Figs. 14 and 15). A few metamyelocytes showed scanty irregularly staining cytoplasm, with irregular

TABLE

MYELOGRAMS IN THIOURACIL NEUTROPENIA

Cell types	Percentage of cell types								
	From counts of 1,000 cells				From counts of 500 cells				
	Case 1*a*	Case 1*b*	Case 1*c*	Case 1*d*	Case 2	Case 3	Case 4	Case 5	Case 6
Myeloblasts	2.5	2.4	0.9	0.5	1.6	1.8	2.0	1.8	0.6
Promyelocytes	3.0	1.5	5.0	4.2	7.6	9.4	7.4	2.0	6.8
Neut. myelocytes	0.6	1.6	8.3	19.6	9.8	2.4	5.8	0.8	6.8
Neut. metamyelocytes	0.1	0.6	2.9	21.3	24.0	23.6	18.0	1.4	24.8
Neut. segmented	0.1		0.1	6.5	8.6	12.2	11.6	3.2	19.2
Eos. myelocytes				0.2	1.6	1.0	1.2		0.4
Eos. metamyelocytes									0.2
Eos. segmented					0.2	1.2	1.0		
Adult basophils				0.1		0.2			
Megakaryocytes	0.1	0.1		0.1			0.2	0.2	0.2
Lymphocytes	23.6	34.2	42.1	23.4	22.8	17.4	6.0	25.4	12.6
Plasmacytes	2.8	2.2	2.0	3.1		0.6	0.8	0.5	1.6
Monocytes	2.5	3.4	0.4	0.7	1.6		0.6	0.6	0.8
Reticulum cells	1.0	0.8	0.1	0.6	0.2	0.2	0.6	0.4	0.2
Cells in mitosis—									
Promyelocytes			0.1	0.1		0.2	0.2		0.2
Neut. myelocytes			0.1	0.1	0.2				
Haemocytoblasts		0.2				0.2	0.4	0.4	0.8
Pronormoblasts	2.0	0.7	0.9	0.4	0.6	1.0	1.2	1.4	1.8
Basophil normoblasts	9.8	5.5	2.7	0.4	1.4	3.2	8.0	7.2	1.4
Polychromatic normoblasts	15.6	13.0	13.2	5.5	5.0	5.0	11.0	16.6	6.0
Orthochromatic normoblasts	15.4	9.5	5.9	2.4	8.6	2.6	12.0	14.8	5.6
Cells in mitosis—									
B. normoblasts	0.3	0.2	0.4	0.1	0.2		0.2		0.2
P. normoblasts	0.1		5.9	0.2				0.8	
Bare nuclei	20.5	24.1	14.6	10.5	6.0	17.2	11.8	22.5	9.8
Myelo-lymphoid ratio	0.3/1	0.2/1	0.4/1	2.2/1	2.3/1	3/1	8/1	0.36/1	4/1
Myelo-erythroid ratio	0.2/1	0.19/1	0.7/1	6/1	3.4/1	4.4/1	1.5/1	0.23/1	4.7/1

outline, vacuolation, and ill-defined or absent granules (Fig. 16).

A further puncture was performed on April 7, 1947, at a time when granulocytes were beginning to reappear in the peripheral blood smears (64 per c.mm.). The sternal marrow (Table, 1*c*) showed evidence of myeloid regeneration, and only one abnormal myeloblast with vacuolated cytoplasm could be seen. Many of the promyelocytes showed abnormal features as described above. Some of the neutrophil myelocytes were abnormally large; the nucleus of some exhibited nucleoli, and the cytoplasm of others, was completely disrupted (Fig. 17). A fresh feature noted in the cytoplasm of metamyelocytes was the coarse granulation; that of the single polymorph was abnormally pale, with irregular outline and devoid of granules (Fig. 18) (120 abnormal myeloid cells were seen).

The final puncture, performed on April 10, 1947, was made when the peripheral granulocyte count was rising rapidly. The smears showed marked evidence of myeloid regeneration (Table, 1*d*). All the myeloblasts appeared normal, and only 39 of the remaining myeloid cells showed any of the abnormal morphological features described above.

Case 2.—Sternal puncture was performed on Oct. 30, 1947, peripheral blood smears showing 1,160 granulocytes per c.mm. There appeared to be a "myeloid maturation defect" (Table, 2). The myeloblast morphology was normal, but 25 of the more differentiated myeloid forms showed abnormalities of structure of the types described in Case 1. Additional features worthy of note were 2 metamyelocytes with abnormally large nuclei (Fig. 19), and one eosinophil myelocyte with vacuolated cytoplasm.

Case 3.—On March 26, 1947, 1,200 granulocytes per c.mm. were seen in peripheral blood smears. The marrow (Table, 3) showed a relatively slight apparent myeloid "maturation defect," while 27 myeloid cells exhibited abnormalities of the types noted in the previous cases (Fig. 20).

Case 4.—The peripheral granulocyte level standing at 854 per c.mm. sternal marrow smears obtained on May 20, 1947, showed an apparent "maturation defect" in the myeloid cells (Table, 4). The normoblastic reaction was expected in view of chronic haematuria (on admission she had 50 per cent Hb, Hellige). Worthy of note was the disrupted cyto-

plasm of two neutrophil polymorphs and two eosinophil polymorphs (Fig. 9); otherwise, the 20 abnormal myeloid cells showed no features not already seen in the other cases.

Case 5.—On June 1, 1947, when the sternal marrow was examined, there were 504 granulocytes per c.mm. in the peripheral blood. Again the Table (5) shows a myeloid "maturation defect," and 27 abnormal myeloid cells showed changes described above.

Case 6.—Sternal marrow puncture was performed on Dec. 1, 1947, when 1,250 granulocytes per c.mm. were seen in the peripheral blood. Marrow smears (Table, 6) showed 10 abnormal immature myeloid cells, 1 myeloblast, 2 neutrophil myelocytes, and 7 premyelocytes with neutrophil myelocytic nuclei. Otherwise there was no new feature.

Sternal marrow examinations were carried out on five thyrotoxic patients in whom no neutropenia occurred during thiouracil therapy. In these, neither the morphological abnormalities, nor the marked myeloid depression described in the six neutropenic cases, were observed.

Discussion

The six cases showed varying grades of neutropenia up to complete agranulocytosis in Case 1. The sternal marrow appeared to contain an excess of lymphocytes in Cases 1, 2, and 5, and of plasmacytes in Case 1, these findings being common in cases of neutropenia and agranulocytosis (Darling and others, 1936). The lymphocyte morphology was normal throughout, no cells of the type described by Downey and Stasney (1936) being evident. There appeared to be no significant increase in reticulum cells and haematogones (haemocytoblasts) such as McGavack and others (1944) found in a case of thiouracil neutropenia. The erythropoietic tissue showed no constant variation from normal, as one would expect (Wintrobe, 1946a). In no case was there a megakaryocytic increase, found by Gessler (1946) in two of his series of nine patients. The latter had been treated with thiouracil, and two had developed neutropenia.

The most striking changes were found in the myeloid series; they were constantly depressed (see Table), and displayed morphological alterations as described above. Gessler (1946) and Limarzi and Ricewasser (1946) have reported a few of these, while some of the other abnormalities have been described previously in cases of agranulocytosis not associated with thiouracil (Rotter, 1925; Jaffé, 1933; etc.). There were obvious discrepancies in the maturation of the nucleus and cytoplasm throughout the series (Plate I, Figs. 1, 8, 11, 13, 17, and 19).

The mechanism of the depression of the bone marrow, and of the leucopenia, has by no means been fully elucidated. Neutropenia appears to depend on the development of hypersensitivity of the white cells to the drug, and other symptoms suggestive of allergy appear from time to time during treatment—for example, skin rashes and drug fevers (Witts, 1936; Plum, 1937; Hadler, 1940; Lesses and Gargill, 1945; Fishberg and Vorzimer, 1945; etc.). Our case of agranulocytosis developed erythema, but none of the cases showed eosinophilia at any stage. Williams and Clute (1944) showed that patients may recover from neutropenia with continued administration of the drug, and desensitization to thiouracil has been noted by several authors (Sprunt, 1944; Rose and McConnell, 1944). However, Williams and others (1944) have shown that human bone marrow is one of the tissues most highly saturated with the drug. They also found that the concentration of thiouracil in the leucocytes was greater than in the erythrocytes. Palmer (1944) supports these findings, and Warren (1945) showed that thiouracil produced a small but significant inhibition of respiration of rabbit bone marrow cells, more especially of the immature myeloid cells. Thus a direct toxic effect of the drug may play a part. It is felt that the morphological abnormalities described above, and affecting even the earliest myeloid cells, and the discrepancies in maturation between the nucleus and cytoplasm strongly support this view. Noteworthy, too, is the chemically close relationship of the thiourea drugs to barbitone or diethylbarbituric acid, which latter occasionally gives rise to agranulocytosis (Watkins, 1933). That the neutropenia does not arise from thyrotoxicosis *per se* has been adequately shown (Lesses and Gargill, 1945), and there is evidence that anoxia does not play an important part (Rosin and Rachmilewitz, 1948).

Whether depressed function of the marrow is due to allergy or to a direct toxic effect and overdosage, or both, actual production of the neutropenia would appear to be explicable by either one or other of the following concepts:

(1) The much favoured "maturation arrest" (Fitz-Hugh and Krumbhaar, 1932; Fitz-Hugh and Comroe, 1933), in which the effect is exerted on the precursors of granulocytes, interfering with their maturation. Thus Rubinstein (1944) performed serial marrow examinations in a case of severe thiouracil neutropenia and came to the conclusion that agranulocytosis was caused by arrest of maturation associated with hypoplasia.

(2) Plum's (1937) evidence favours the view that the bone marrow in agranulocytosis goes through a number of stages. The first is a reduc-

tion of granulocyte precursors, soon followed by a loss of mature and then immature granulocytes. Recovery is heralded by the reappearance of granulocyte precursors, the picture then being one of so-called " maturation arrest." Later, the mature granulocytes return. (It is of interest to note that Jaffé, 1933, described three cases of idiopathic agranulocytosis, and one following anti-luetic treatment. The femoral marrow at autopsy was hyperplastic, the granulopoietic tissue taking an active part. He thought, therefore, that in some instances the agranulocytic catastrophe was preceded by proliferation of young myelocytes.) The work of Braun (1944), of Dameshek (1944), and of Sikkema and others (1946) supports this second view.

Our cases would also seem to favour the Plum concept, both from the evidence of a direct toxic effect already mentioned and from that afforded by the differential marrow counts in the Table. There is evidence of proliferation of earlier myelocytes in most cases, and in Case 1 this preceded the development of the more mature granulocytes. Also in favour are the large number of " bare nuclei " showing the general morphology of myeloblastic or premyelocytic nuclei. We must remember, however, that destruction of some of the granulocytes may occur in the circulating blood (Lawrence, 1941).

Fisher (1947) pointed out that neutrophil leucopenia occurred in his series of cryptogenic acquired haemolytic anaemias only in those cases with liver dysfunction. In retrospect, he noted that neutropenia was the only early and constant herald of liver dysfunction. It is interesting to speculate about the hepatic role in thiouracil neutropenia. The liver is affected in hyperthyroidism. There is often passive congestion, and degenerative changes are common. Fatty change, acute necrosis, both focal and central, and subacute toxic atrophy with the development of cirrhosis occur frequently. Liver function tests often show marked impairment (Weller, 1930 ; Beaver and Pemberton, 1933 ; Boyd, 1943). In addition, thiouracil therapy may be associated with jaundice and hepatic enlargement (Kahn and Stock, 1944 ; Paschkis, 1944 ; Sloan and Shorr, 1944 ; Lesses and Gargill, 1945 ; Linnell and others, 1946 ; Livingston and Livingston, 1947). Case 5 had hepatomegaly, but all the generally accepted liver function tests were normal, except the galactose tolerance test, which gave a value of 4.5 g. excreted in five hours after 40 g. had been taken by mouth. Case 3 showed a similar result, and a serum alkaline phosphatase of 19 K-A units. Otherwise, the usual liver function tests were normal throughout our series. This latter does not support the view that liver function has much to do with thiouracil neutropenia.

Peripheral blood films in the six cases showed varying grades of degeneration of some granulocytes. Some had a pyknotic nucleus, or a pale nucleus with no cytoplasm but surrounded by granules. Vacuolation of the cytoplasm, abnormal bluish staining, and coarse and fine poorly staining granules were seen. In no case was a leucocytosis detected on recovery (cf. Reznikoff, 1938 ; Lesses and Gargill, 1945 ; Kneedler, 1946 ; etc.).

Bacteriological cultures of the marrow under aerobic (blood agar, glucose broth) and anaerobic (blood agar and Robertson's meat medium) conditions were carried out in Cases 1, 3, 4, and 5. After fourteen days' incubation they remained sterile. In each case the marrow sample was taken before the commencement of penicillin therapy. These findings support the view that the neutropenia is not primarily due to an infection (Wintrobe, 1946b).

Our cases (see appendix) confirm the fact that no general rules will guarantee freedom from serious haematological reactions. Neutropenia was seen at very different dosage levels, and after short and prolonged periods of administration. Clinical symptoms are not reliable as an indication of the blood picture ; thus Case 5 has continued over the past year free from symptoms, with a granulocyte count remaining at about the 1,000 per c.mm. level.

Summary

The sternal marrow pictures of one patient who developed agranulocytosis and of five who developed neutropenia while under treatment with thiouracil derivatives are described, with particular reference to numerical and morphological changes in the myeloid series, which was constantly depressed.

The mechanism of this depression is discussed, and evidence in favour of a direct toxic effect of the drug is given. The morphological abnormalities described, affecting even the earliest myeloid cells, and the discrepancies in maturation between the nucleus and cytoplasm strongly support this view.

The mode of production of thiouracil neutropenias is better explained by the Plum (1937) concept than by that of " maturation arrest."

The liver function tests performed on these cases do not uphold the view that liver function has much connexion with thiouracil neutropenias.

Bacteriological cultures of the sternal marrow were sterile in four cases examined by suitable methods. This negatives infection as a primary cause of the neutropenias.

It was confirmed that no general rules will guarantee freedom from serious haematological reactions, and clinical symptoms are not reliable as an indication of the blood picture.

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Appendix

Case 1.—A married woman aged 62 years was admitted to hospital with typical thyrotoxicosis and congestive heart failure. Methyl thiouracil, 0.2 g. three times a day, was begun on March 12, 1947. The granulocytes fell from 2,120 on March 5 to 1,122 per c.mm. on March 12, after which a satisfactory level was maintained until March 31, when a value of 1,188 per c.mm. was found. On March 24 thiouracil therapy had been stopped for twenty-four hours in view of a transient mild sore throat. The granulocytes fell to 64 per c.mm. (total leucocytes 3,200 per c.mm.) by April 3; the drug was again stopped, and the first sternal puncture performed. Treatment with penicillin, intravenous pyridoxine, and intramuscular pentnucleotide was begun, her only fresh symptom being a slight sore throat.

A few hours after the first sternal puncture on April 3 she complained of malaise, headache, pains in her back and limbs, and a sore throat. At 6 p.m. she had a rigor, her temperature rising to 101° F. She appeared acutely ill, with flushed facies and coated tongue. Her fauces were injected, but no exudate was evident. Slightly enlarged, tender, tonsillar lymph nodes were palpated. There was no splenic or hepatic enlargement, and no bone tenderness, icterus, or skin rash.

The following day the temperature remained intermittent, and the peripheral granulocyte level stood at 60 per c.mm., with a total leucocyte count of 3,000 per c.mm. At 10.40 p.m. she experienced a rigor, this being thrice repeated on April 5, on which day she developed generalized erythema, and had a leucocyte count of 2,800 per c.mm., no neutrophil polymorphs being evident in peripheral or marrow films.

The complete neutropenia was maintained on April 6, and she had a rigor at 10 p.m., which was repeated the next day at 3 p.m., when her peripheral blood contained 64 granulocytes per c.mm. Another sternal marrow examination was made on April 7.

Marked clinical improvement took place on April 8, this being reflected in her peripheral blood, which showed 704 granulocytes (64 neutrophil myelocytes, 96 metamyelocytes, and 544 polymorphs), out of a total of 3,200 leucocytes per c.mm. The following day no neutrophil myelocytes were seen, and the granulocyte level rose to 924 per c.mm. The final marrow smears were examined on April 10, 1947.

Thereafter, the patient's leucocyte counts were satisfactory, and methyl thiouracil 0.2 g. twice a day was recommenced on April 29. Agranulocytosis which, apart from a moderate rise of temperature, was symptomless developed suddenly on May 19. The drug was stopped and she recovered on suitable treatment.

Eight months later, in view of increasing toxicity, and the previous reactions to methyl thiouracil, thyroidectomy was performed after suitable preoperative treatment. The patient died a few hours later in a thyroid crisis.

Case 2.—In 1941 a married woman aged 45 years had a subtotal thyroidectomy for typical thyrotoxicosis. In view of recurrent toxic symptoms, methyl thiouracil 50 mg. per day was begun on Aug. 8, 1946. The dose was reduced to 25 mg. daily on Oct. 31, being raised again to 50 mg. on March 27, 1947. Her granulocyte count was satisfactory until Oct. 24, 1947, when a leucopenia (2,600 per c.mm.) and neutropenia (936 per c.mm.) were found. The granulocyte level varied between 936 and 1,160 per c.mm. over the next few symptomless days, thiouracil therapy being stopped and penicillin and pyridoxine exhibited on Oct. 26, 1947. Sternal puncture was performed on Oct. 30, 1947.

Case 3.—A married woman aged 38 years was admitted to hospital with auricular fibrillation and congestive heart failure due to primary thyrotoxicosis. Propyl thiouracil 50 mg. three times a day was given for fourteen days, 25 mg. three times a day for the next five days, and then 25 mg. twice daily for seventeen days, after which (March 20, 1947) 25 mg. daily sufficed. Her total leucocyte count varied between 3,000 and 3,600 per c.mm. from March 17 until March 26, when her sternal marrow was examined. Previous counts had been normal, and she never had symptoms attributable to neutropenia.

Case 4.—A married woman aged 43 years came into hospital on April 28, 1947, suffering from thyrotoxicosis and left pyonephrosis. Her leucocyte count was 2,400 per c.mm. with 1,584 granulocytes per c.mm. Propyl thiouracil, 50 mg. three times a day, was given, and she developed more marked leucopenia, which was symptomless and persisted for four days before her sternal marrow was examined on May 20. On this date the peripheral blood showed 1,400 leucocytes and 854 granulocytes per c.mm.

Case 5.—A man aged 53 years, a case of primary thyrotoxicosis, was given methyl thiouracil 200 mg. per day for one month, and then 100 mg. daily for three and a half months, followed by 25 mg. daily for one year. He then developed leucopenia (3,800 per c.mm.) due to neutropenia (418 granulocytes per c.mm.) associated with palpable enlargement of liver and spleen on May 31, 1947. He had no pyrexia, sore throat, skin rash, adenitis, icterus, bone tenderness, or anaemia, the neutropenia being symptomless.

Case 6.—A married woman aged 49 years had had a thyroidectomy four years previously for toxic goitre. She relapsed, and there was a concomitant *B. coli* pyelonephritis. On admission on Nov. 28, 1947, she had hypochromic normocytic anaemia (Hb, 67 per cent Hellige), and leucopenia (2,000 per c.mm.) and neutropenia (1,220 granulocytes per c.mm.). The following day methyl thiouracil, 200 mg. twice a day, was begun, and her marrow was examined on Dec. 1, there having been no symptoms attributable to the neutropenia.

LEGENDS FOR PLATE I

(All the photomicrographs are Leishman-stained, and the magnification is × 1,000)

FIG. 1.—Myeloblast showing an irregularly shaped nucleus and irregularly staining cytoplasm with interruptions in the continuity of its outer margin.

FIG. 2.—Degenerating myeloblast showing scanty, irregularly staining cytoplasm, with irregular outer margin, and pseudopodium-like formation.

FIG. 3.—Large promyelocyte with a few coarse cytoplasmic granules.

FIG. 4.—Large promyelocyte showing a cytoplasmic vacuole, and irregularity of cytoplasmic staining and outline.

FIG. 5.—Promyelocyte showing irregularly staining cytoplasm, which contains coarse granules and a vacuole.

FIG. 6.—Promyelocyte showing irregularly staining cytoplasm and coarse granules.

FIG. 7.—Promyelocyte showing irregularly staining cytoplasm with irregular outer margin and pseudopodium-like formation. Some coarse cytoplasmic granules are seen.

FIG. 8.—Nucleus only and promyelocyte, showing morphological similarities. The promyelocyte also shows an irregularly shaped nucleus, and ill-defined granules, which seem to fuse with the cytoplasm.

FIG. 9.—Degenerate polymorphonuclear cell showing a stage in development of "nucleus only."

FIG. 10.—*N. myelocyte* showing ill-defined granules apparently fusing with the cytoplasm.

FIG. 11.—Myeloblast showing partially segmented nucleus.

FIG. 12.—Promyelocyte with ill-defined granules apparently fusing with the cytoplasm.

FIG. 13.—Promyelocyte with irregularly shaped nucleus and granules as in Fig. 12.

FIG. 14.—*N. myelocyte* showing vacuolated cytoplasm with absent granules.

FIG. 15.—Large *N. myelocyte* with coarse cytoplasmic granules.

FIG. 16.—Metamyelocyte with scanty irregularly staining vacuolated cytoplasm and ill-defined granules.

FIG. 17.—*N. myelocyte*. The nucleus contains nucleoli. The irregularly staining cytoplasm has an uneven outer margin and contains coarse granules.

FIG. 18.—*N. polymorph* with abnormally pale cytoplasm, which has an irregular outline and is devoid of granules.

FIG. 19.—Metamyelocyte with abnormally large nucleus and ill-defined cytoplasmic granules.

FIG. 20.—Eosinophil myelocyte with disrupted cytoplasm.

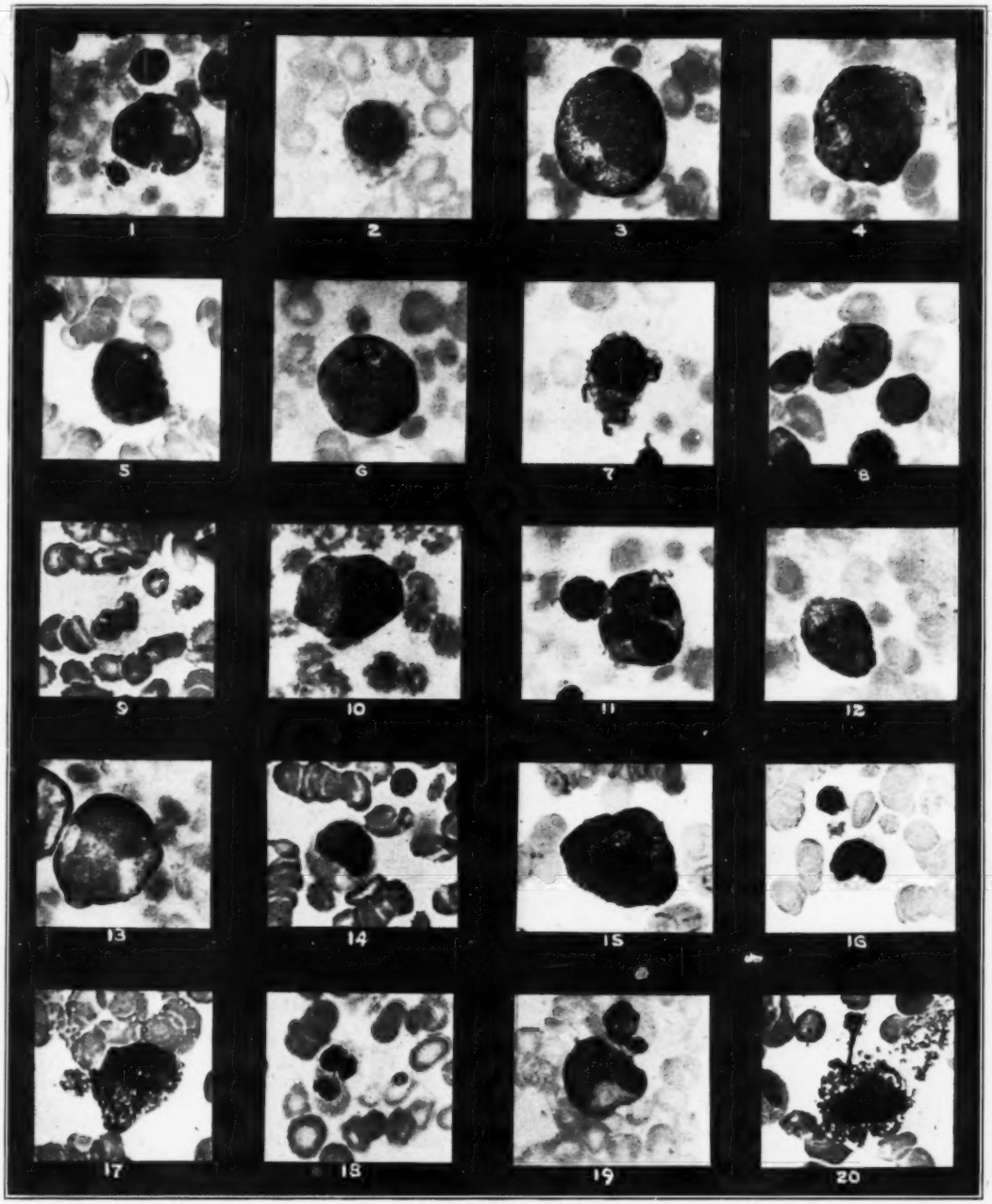

PLATE I

J. clin. Path. (1948), **1**, 302.

CONGENITAL DEFECTS FOLLOWING RUBELLA

REPORTS OF TWO CASES, ONE OF WHICH SHOWS A HITHERTO UNDESCRIBED LESION

BY

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The association of maternal rubella in the third month of pregnancy with ocular defects in the infant was first noticed in New South Wales by Gregg (1941). Subsequently (1946) the matter was investigated by Swan and his collaborators in South Australia. Further investigations have been made in the United States and in this country. By now there have also been reports of maternal rubella preceding the following defects in the infant: congenital abnormalities of the heart, abnormal size of the head, deafness, feeding difficulties, subnormal weight.

Apart from records by Swan (1944) very few post-mortem observations have been made. His "Final Report" (1946) mentions "heart disease" in a number of the cases, but there are no further post-mortem records. We have been unable to trace any such records in this country. The already voluminous literature on the subject deals in the main with case reports, probabilities, and theories of mechanism.

The purpose of the present paper is to record two further instances in which post-mortem examinations were made and to discuss relevant findings.

Case Reports

Case 1.—An infant was born to a primipara of 26 years. It was known that the mother had had rubella when ten weeks pregnant. The delivery was normal, but the child lived for only fifteen minutes.

Post-mortem findings.—Full-term female child weighing 6 lb. There were no external abnormalities. The body was radiographed, but no bony abnormalities found. The eyes were bisected and found normal. Cranium and brain were also normal.

The thorax and abdomen were anatomically grossly abnormal (Plate II). The small bowel and the left lobe of the liver (1) were seen to be in the thoracic cavity. The heart (2) and lungs were deviated to the right, and both lungs were much compressed. A thin edge of both lobes of the left lung ran diagonally across the thorax (3) from the apex of the cavity to the lower right-hand corner, where it met the displaced heart and pericardium. A large thymus gland (4) covered the upper portion of the pericardium and filled the whole of the superior mediastinum.

The right muscular portion of the diaphragm was well formed (5, 16). The posterior muscular portion of the left half of the diaphragm was present (17), but there was no anterior two-thirds of the left half of the diaphragm (6). Through the gap thus made had herniated the left lobe of the liver, the stomach and nearly all the small intestines, the spleen, the pancreas, and in an extraordinary fashion the bulk of the colon.

The caecum and appendix were to the left of the mid-line in the epigastric region (7). The ascending colon (8) traversed the left side of the liver and stomach to reach the top of the left thoracic cavity. It descended (9) antero-superiorly to the spleen (21), which itself lay on the left diaphragm, to come into more normal lateral relationship with the left kidney (13).

The pelvic colon (10) was unnaturally mobile and ran from the left iliac fossa upwards and then across in a wide sweep to the right iliac fossa, where it entered the pelvis minor on the right side. In its sweep it embraced the left ovary and fallopian tube which lay on its mesentery to the right side of the mid-line. The uterus (11) was rotated through 30 degrees, so as to face to the left.

The heart (2) was of normal size and shape, although it lay in the right thoracic cavity.

There was a large patent inter-auricular septum, but no interventricular communication. The pulmonary artery was of average size, both in length and diameter. The ductus arteriosus was more prominent and of wider lumen than the aorta. It was 2 cm. in length from the origin of the left pulmonary artery to its junction with the aorta beyond the left subclavian artery. It formed a complete right angle with the descending aorta, and ran almost parallel to the ascending aorta in the manner shown (see diagram).

In its descending and abdominal portions the aorta appeared to be normal in length, lumen, and anatomi-

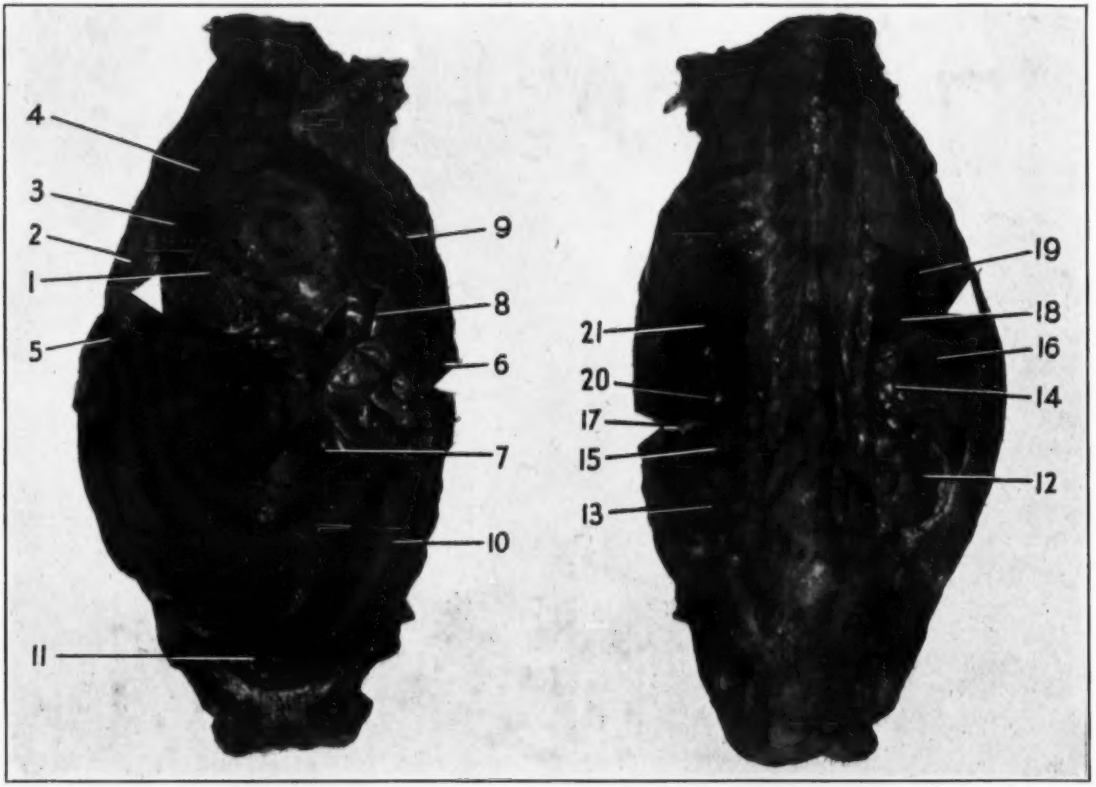


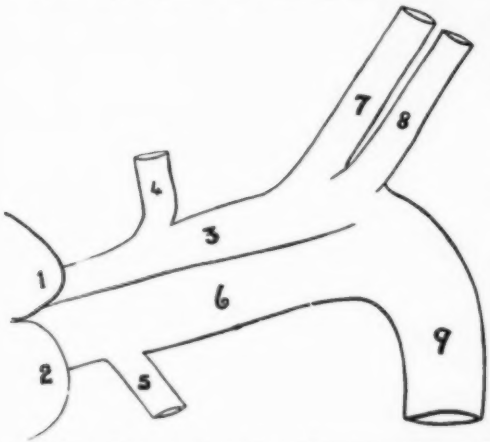
PLATE II.—(*Left*). Case 1 viewed from the front. The area between the white triangular pointers indicates the gap in the diaphragm. Key = (1) left lobe of liver ; (2) heart ; (3) anterior edge of left lung ; (4) thymus ; (5) right half of diaphragm ; (6) rudimentary left diaphragm ; (7) caecum and appendix ; (8) ascending colon ; (9) descending colon ; (10) pelvic colon ; (11) uterus.

(*Right*). Posterior view. Key = (12) right kidney ; (13) left kidney ; (14) right adrenal ; (15) left adrenal ; (16) right diaphragm; (17) left diaphragm ; (18) lower lobe right lung ; (19) middle lobe right lung ; (20) descending colon ; (21) spleen.

cal position. Although the trachea was anatomically displaced to the right, it was normal in size and shape.

The oesophagus maintained normal relationship with the aorta until its emergence at the diaphragm. There was no diaphragm to the left and anteriorly, although there was a thin muscular slip to the left posteriorly, and a fully formed diaphragm to the right.

The stomach lay along the left border of the dorsal vertebrae and it was of normal size and shape. No abnormality of pylorus or duodenum was observed.



Relations between the aorta, pulmonary artery, and ductus arteriosus in Case 1. Key = (1) right auricle; (2) left auricle; (3) ascending aorta; (4) innominate artery; (5) left pulmonary artery; (6) ductus arteriosus; (7) right carotid artery; (8) subclavian artery; (9) descending aorta.

The kidneys (12 and 13) were normal anatomically and histologically. So were the ureters and bladder. The adrenal glands (14 and 15) were normal in size, shape, and position. The pancreas was displaced upwards into the left thoracic cavity. The spleen (21) lay on the thin slip of left diaphragm within the chest wall.

Case 2.—A male baby of three days was admitted with his mother to hospital because of marked oedema of the lower half of the body, jaundice, vomiting, and loose stools. This infant, the second child, was born at home. Delivery had been normal, and the weight at birth was 6 lb. 9 oz.

Two events had interrupted the otherwise normal course of pregnancy. Towards the end of the third month of the pregnancy the mother had contracted rubella. The infection was mild and did not appreciably affect her general condition. At four months she was threatened by abortion. After a few days' rest in bed the danger passed, and the further course of the pregnancy remained uneventful.

The first child, aged 15 months, was well. The parental history did not reveal anything relevant.

Clinical findings.—The infant was normal, well developed, slightly jaundiced, with extensive oedema of feet, legs, scrotum, and genital area, sacral region, and lower abdominal wall. Heart, lungs, abdomen, urine, and blood were all normal. Bilateral cataracts were noticed a few days after admission. Apart from a large anterior fontanelle the skull was normal.

In the course of the following week oedema subsided and the jaundice cleared completely; the infant took the breast well and started to gain weight. On the tenth day after admission mother and baby left hospital.

Fourteen days later the infant, now artificially fed, developed fulminant bronchopneumonia and parenteral gastro-enteritis, and was readmitted in a moribund condition. He died within thirty-six hours.

Post-mortem findings.—The body appeared normally formed but was small. There was slight dehydration. There was bilateral congenital cataract. Out of deference to the parents the eyes were not removed. The cause of death was bilateral pneumonia, more advanced in the right lung. The heart was typically "sabot" shaped. There was dilatation of the right chambers. The ductus arteriosus was widely patent, its lumen not much less than that of the pulmonary artery, although the wall was thinner. Its endothelium was smooth, unwrinkled, and directly continuous with that of the larger arteries. Microscopic examination did not show any evidence of obliterative endarteritis in the patent ductus arteriosus. No abnormality was found in the glomeruli of the kidneys.

Discussion

The association of rubella in the first three months of pregnancy with congenital defects in the foetus has been noted by Clayton-Jones (1947), Hughes (1945), and Hope-Simpson (1944) amongst others in this country. Most investigations have been made by tracing histories from children showing congenital defects to a maternal infection (Swann, 1944; Clayton-Jones, 1947; Conte and others, 1945; Erickson, 1944).

A different line of investigation was followed by Fox and Bortin (1946). They reviewed the notifications of rubella in a certain community over a period of three years. The total number of cases in all classes of people was more than 22,000. Of 152 married women notified, eleven were pregnant. Nine of these were in the first four months of their term. In these cases there was one twin birth, one blue baby with hydrocephalus which receded spontaneously, and one stillborn hydrocephalic. The remaining children were normal.

Parsons (1946a) states that causal relationship between maternal rubella and congenital defects has not been definitely proved, and in a further paper (1946b) he discusses some of the difficulties of the theory. Swan (1944) describes patency of the ductus arteriosus in his series of babies coming

to necropsy. Gregg is quoted (Parsons, 1946b) as finding forty-four cases of congenital heart disease out of seventy-eight affected infants.

Other abnormalities described have been mental retardation, microcephaly, mongolism, hypospadias, talipes equino-varus, and synostosis of radius and ulna or tibia and fibula.

Congenital diaphragmatic hernia has not hitherto been described in the literature as one of the developmental defects occurring in association with maternal rubella.

The diaphragmatic hernia in Case 1 differs from the common form, in which all diaphragmatic segments are present but a patency exists between the pleural and peritoneal cavities. This pleuroperitoneal hiatus, or foramen of Bachdalek, may vary in size, but it is surrounded by diaphragmatic elements. Abassy has quoted Dickson as saying that diaphragmatic hernia is seen in one case out of each hundred routine gastric radiographs.

In this case there was a total absence of the anterior two-thirds of the left half of the diaphragm. There was, however, a small muscular slip to represent the posterior third of the diaphragm.

Developmentally the diaphragm arises in four main parts: a ventral, a dorsal, and a right and a left lateral. The ventral part is formed from a septum transversum which is gradually differentiated into a caudal, an intermediate, and a cephalic part. It is from the intermediate portion that the ventral part of the diaphragm is formed. The dorsal portion of the diaphragm arises from the mesoderm of the dorsal mesentery of the foregut. The two lateral portions grow towards the median plane until they fuse with the dorsal portion.

The present appearance of the diaphragm would suggest that there had been a failure of growth of the left lateral portion and of the left half of the ventral portion. It resulted in a very rudimentary left half of the diaphragm, and this gross diaphragmatic hernia.

The time of formation of the diaphragm from its various elements is from the eighth week onwards, after the pericardial cavity has already been shaped. The inter-atrial and interventricular septa begin to develop between the fourth and sixth weeks. The former is the result of the coalescence of the primary and secondary septa. Their fusion is partial and leaves the foramen ovale. The valve of the foramen is formed in the fourth month. The interventricular septum arises in the lower part of the primitive ventricle, and grows upwards to meet the endocardial cushions and the septum developing in the bulbus arteriosus. The lower part is complete about the eighth week, and the septum of the bulbus arteriosus a few days earlier.

In Case 1 the mother was stated to have suffered from rubella when ten weeks pregnant. The time of formation of the diaphragm is about the eighth week. The possibility that the maternal disease and the time of formation of the affected part coincided is not to be lightly dismissed.

In Case 2 the mother suffered from rubella at about the third month. The ductus arteriosus forms about the sixth week ; the valve of the intra-atrial septum about the sixteenth week ; the inter-atrial septum about the eighth week. Thus the infection would have had ample opportunity to interfere with the development of the cardiac septa.

Parsons (1946b) has canvassed the possibility of strain differences, as well as emphasizing the difficulties of diagnosis. This is a point which most authors in this country stress. Some support for the theory is given by Conte and others (1945), who found that the incidence of rubella in the appropriate months of pregnancy in mothers of children with congenital abnormalities was ten times the expected rate for women of the child-bearing age of the population at large.

Swan and others (1946) do not deal only with rubella as an antecedent of congenital defect. They list cases in which influenza, scarlet fever, herpes zoster, varicella, and mumps, together with one of "pustular rash," were antecedents.

Summary

Post-mortem records of two infants with congenital malformations following maternal rubella are described. Case 1 presented an unusual form of congenital diaphragmatic hernia and a widely patent ductus arteriosus. Case 2 had bilateral congenital cataract and a wide patency of the ductus arteriosus.

We wish to thank Mr. G. G. Alderson, under whose care Case 1 was born and who has encouraged publication of the case ; Dr. J. Wearing for taking *x*-ray photographs ; and Mr. T. L. Skuse and Mr. G. Wright for technical assistance.

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STUDIES ON TETANUS PROPHYLAXIS

BY

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Before the second world war several investigators had demonstrated the possibility of actively immunizing man against tetanus (Ramon and Zoeller, 1927; Jones and Moss, 1937; Marvell and Parish, 1940; Wolters and Dehmel, 1940). During the war this method was tested on a large scale, for in the allied armies prophylaxis against tetanus was based almost entirely on active immunization. Reports so far clearly indicate its value and its superiority to serum prophylaxis (Boyd and MacLennan, 1942; Long, 1946; Ramon, 1946).

Comparison between Active and Passive Immunity

The amount of antitoxin per ml. of serum required for protection against tetanus after active immunization of man can be estimated in at least two different ways: (1) by determining the level of antitoxin capable of protecting actively immunized animals against artificial infection; (2) by consideration of the amount of antitoxin known to be generally effective in passive prophylaxis against tetanus in man.

The first method takes no account of the difference in susceptibility to tetanus toxin between man and animal nor to the difficulties in estimating the amount and rate of toxin production in the artificial infection or its relation to that occurring in naturally sustained injuries. Since in the pathogenesis of tetanus the intoxication predominates and the infection plays a minor part, the total amount of antitoxin available as well as its concentration must be of great importance, and no direct conclusions should be drawn from experiments on small laboratory animals.

Accordingly we adopted the second method, which depends on the established fact that 3,000 I.U. of antitoxin gives effective protection against tetanus for eight to twelve days after injection.

Titrations of tetanus antitoxin in serum after passive immunization have been reported by Sneath (1934) and Gold (1941). Three days after injection of 3,000 I.U. the serum antitoxin level lay between 0.2 and 0.5 I.U. per ml.; a week after injection it lay between 0.02 and 0.2 I.U. per ml.

In order to estimate the passive immunity the antitoxin titre in the serum of a number of outpatients was determined at varying intervals after the intramuscular injection of 3,000 I.U. of unrefined tetanus antitoxin, which was given as for routine prophylaxis after accidental injuries. (This work, begun in 1943, is referred to in a paper by Ericsson and others, 1944.) The titrations were carried out by a micro-method, a modification of the standard method used for routine purposes in the manufacture of the commercial products of this laboratory.

Three times the quantity of tetanus toxin, which in titration against international standard serum has been found to correspond to 0.005 I.U., is mixed with 1.5 ml. of undiluted serum or serum dilution. One third of the mixture is injected into each of two 16-g. mice. If both survive, the serum sample or dilution is considered as containing more than 0.005 I.U. per 0.5 ml.

The method thus allows the determination of a minimum concentration of 0.01 I.U. tetanus antitoxin per ml. The results for 56 bleedings are given in the Figure. On the Y axis the absolute

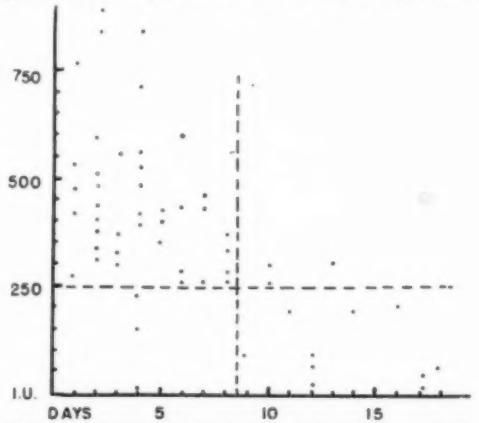

FIGURE.—Showing the total amount of antitoxin available in serum after the injection of 3,000 I.U.

amount of antitoxin available in the patient's circulation is plotted, on the assumption that the patient has 40 ml. of serum per kg. of body-weight.

The values obtained vary considerably from one patient to another. During the first eight days 40 out of 43 values lie above 250 I.U.; during the following days only 3 out of 13 exceed that value. Having assumed that passive immunity is effective for eight days, we concluded that satisfactory protection against tetanus requires 250 I.U. of circulating antitoxin, corresponding to 0.1 I.U. per ml. serum in a 65-kg. man. Such a titre should offer reasonable protection against tetanus in man, and if the same titre is achieved by active immunization a broad safety margin is gained through the inherent superiority of active immunity.

There was no opportunity of titrating serum antitoxin in patients given a second injection of antitoxin. If the second injection is from an animal species different from the first, the rate of loss of antitoxin should not differ from that in the first injection. The opportunities of using serum from different animal sources are few, and consequently for second passive immunization serum from the same species often has to be used. Though the risks of disagreeable and even dangerous reactions after such a procedure have long been recognized, less attention has been paid to the reduced efficacy of the second dose. According to general rules of immunology, one would expect the rate of loss of antitoxin after the second dose of heterologous serum from the same species to be greater than that after the first dose, since a greater capacity for eliminating heterologous serum must be present at the second injection. The practical importance of this has been pointed out by Ramon and his co-workers (1939), but there are very few direct observations bearing on this question. Since the estimation of the efficacy of passive immunization is based on primary injections, the comparison between active and passive prophylaxis should be based on primary injections of antitoxic serum.

Active Immunization

The adsorbed toxoid used in these investigations is that produced regularly at the State Bacteriological Laboratory. It is prepared according to a method primarily elaborated for the production of adsorbed diphtheria toxoid. This method has already been described (Ericsson, 1946).

The method involves precipitation of toxoid with trichloracetic acid and resolution of the precipitate in Na_2HPO_4. The addition of $AlCl_3$ to this solution gives a precipitate of $AlPO_4$ to which the active substance is adsorbed. No washing of this precipitate is done. The difficulty of obtaining accurate flocculation of the purified preparation prevents an exact determination of the amount of active substance in the final product. However, in view of the titre of 10 to 15 Lf of the raw material, the content of the final product may be estimated at 30 Lf/ml. Guinea-pigs weighing 250 to 300 g., to which 1 ml. of toxoid is given (the dose designed for human use), will give a titre of more than 1 I.U./ml. of serum in more than half the animals after six weeks.

Ninety-seven healthy men, 20 years of age, who had not received an anti-tetanus inoculation were used in the following experiment. A preliminary test of every fourth person failed to show the presence of naturally occurring tetanus antitoxin in any serum. A single injection of tetanus toxoid was given to each man, followed 6 months later by a booster dose. Table I shows the titres of antitoxin achieved.

TABLE I

SHOWING THE RESULTS ACHIEVED BY ACTIVE IMMUNIZATION

Immunization	Number of patients with the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01–0.1	0.1–1.0	>1.0	Not tested
No immunization ..	25				72
1 month after single dose	16	64	17		
6 months after single dose	1	23	43		30
4 days after booster dose	1	8	68		20
8 days after booster dose	1			76	20

Bleedings one month after the injection show that it is impossible to obtain, within this space of time, an immunity comparable to that produced by passive immunization. The bleedings performed five months later disclosed a marked increase in the immunity, proving that the antigenic stimulus by adsorbed toxoid had continued for more than a month. These experiments on man confirm the findings of Faragó (1935), who, when immunizing guinea-pigs, showed that the depot of adsorbed toxoid retained its antigenic properties for thirty-eight days.

The immunity brought about by active immunization with toxoid is inferior even after one month to the best result achieved by serum prophylaxis. Therefore primary active immunization is of no value in the case of an injury unless healing is likely to be so protracted that there is a risk of tetanus even after one month.

The production of antitoxin following the booster dose was of quite a different magnitude and appeared much more rapidly than the antitoxin production after the primary injection (Glenny and Südmersen, 1921). Within four days, that is, within the probable incubation period of tetanus, the booster dose gives an immunity that equals that of the usual passive prophylaxis and, during the following days, surpasses the level of passive immunity. In a person who has already been subjected to basal active immunization a booster dose of toxoid may thus be substituted for the passive prophylaxis.

The results obtained from these studies indicate that the adsorption of the active substance on the precipitate is not so complete as to impair the effectiveness of the toxoid administered as a booster dose. These experiences seem to contradict Miller and Humber's (1943) results, according to which fluid toxoid and not an alum-precipitated one should be used for the booster dose. However, the difference may be explained by the different method employed for the production of the Swedish toxoid. The alum precipitation method usually includes a thorough washing of the precipitate, and it may be assumed that all the activity present is fixed to the precipitate. According to our method the precipitate of $AlPO_4$, the formation of which seems to be the main feature of both the methods, is obtained through the addition of $AlCl_3$ to the solution of $NaPO_4$. Since the purification takes place at an earlier stage in the procedure, and no irritating products are formed by the precipitation, no washing is necessary. Thus the method gives a certain amount of active substance in the supernatant fluid. The difficulty of obtaining accurate flocculations of the toxoid prevents, as already stated, exact determinations. In diphtheria toxoid prepared according to the same method, about one-fourth of the active substance remains in solution, and approximately the same proportion may be assumed to remain in tetanus toxoid. The fact that a certain amount of the active substance of our adsorbed toxoid remains in the solution should be regarded as an advantage, rendering it suitable for the booster dose as well as for the primary injection.

Combined Passive and Active Immunization

Since active immunization is of no immediate value to an injured individual not previously subjected to basal immunization, and as we had to take into account the possible use of serum prophylaxis in the future, it was essential to examine the possibility of attaining a basal active immunity simultaneously with the application of serum prophylaxis.

Investigations performed by Otten and Hennemann (1939) on guinea-pigs, and by Cooke and Jones (1943) on man, have established that passive immunization impairs the development of active immunity.

Preliminary experiments on guinea-pigs were made with our toxoid. Nineteen animals were injected simultaneously but into different sites with 1 ml. of toxoid and 3,000 I.U. of antitoxin (horse). Bleedings were taken on the fourteenth, twenty-eighth, and forty-second days. A control group of six animals was subjected to injections with toxoid only, and the animals were bled on the forty-second day. The results are shown in Table II.

TABLE II

SHOWING THE RESULTS ACHIEVED BY COMBINED PASSIVE AND ACTIVE IMMUNIZATION OF GUINEA-PIGS*

Test animals	Number of animals with the following amount of antitoxin in serum (I.U./ml.)			
	<0.01	0.01–0.1	0.1–1.0	>1.0
Test animals:				
14th day		3	7	9
28th ,, ...		19		
42nd ,, ...	13	6		
Control animals:				
42nd day.. ..			1	5

* The test animals were given 1.0 ml. of toxoid and 3,000 I.U. of antitoxin simultaneously. The control animals were given 1.0 ml. of toxoid only.

They clearly prove the inhibition of active immunization by the heterologous passive immunity, brought on by the simultaneous injection of antitoxic horse serum.

Clinical trials have also been carried out. Since the beginning of 1944 all the patients at the out-patients department of the surgical clinic of the Caroline Hospital, Stockholm, suffering from injuries suspected of being infected with tetanus, were given 1 ml. of toxoid subcutaneously and 3,000 I.U. of horse serum intramuscularly. Two to three years later, seventy of these patients were examined to determine their antitoxin serum titres and the effect of a booster dose of toxoid. At the first examination, bleedings were made for titration of antitoxin in serum, and at the same time a booster dose of 1.0 ml. of toxoid was administered. The effect of the booster dose was determined by further serum titrations four to nine days later. Only thirty-six patients reported for the second control examination. The results are indicated in Table III.

Out of seventy persons tested, thirty-two showed direct evidence of residual immunity as demonstrated by a measurable titre of antitoxin in serum

TABLE III

SHOWING THE REMAINING TITREABLE IMMUNITY IN PATIENTS TWO TO THREE YEARS AFTER COMBINED PASSIVE AND ACTIVE IMMUNIZATION AND THE EFFECT OF A BOOSTER DOSE

	Number of patients showing the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01–0.1	0.1–0.5	0.5–2.0	>2.0
Before booster dose ..	38	27	1	1	3
4–5 days after booster dose	4	5	1		
6–9 days after booster dose	1	2	7	7	9

two to three years after combined active and passive immunization, but only five of them had a satisfactory immunity according to our requirements. They did not show any definite rise in antitoxin content of serum four to five days after the booster dose, but six to nine days after there was a marked rise. In only one case out of twenty-six was the response inadequate.

These investigations show that the combined active-passive immunization procedure has an effect that may still be demonstrated two to three years later by direct titration of the antitoxin content of serum or by the reaction following a booster dose. The antigenic stimulus remains, therefore, even after the disappearance of the passive immunity. In accordance with this finding a single injection of toxoid, even though this be administered together with the usual prophylactic dose of serum, produces a basal immunity, and in the event of another injury a booster dose of toxoid without antitoxin will give adequate protection. Although we do not in any way question the results of Cooke and Jones (1943), who worked with much higher doses of serum, our results show that the dose of serum generally used for prophylaxis does not notably impair the effect of our toxoid.

The basal immunity is also of direct practical importance in the prophylaxis of tetanus following minor injuries which are not treated by a physician. Experience has shown that many cases of tetanus occur after such injuries, and that certain occupations carry a heavy risk in this respect. The primary injection makes a small amount of antitoxin available for a long period. This antitoxin neutralizes the toxin initially produced ; the latter acts as a booster dose and causes a further rise in the antitoxin titre. Wolters and Dehmel (1940) showed in man that the quantity of toxin which may be produced after a naturally sustained injury actually has a considerable antigenic effect in an already actively immunized organism. In view of present knowledge a further condition must be stipulated : that there should be no passive immunity present that might interfere with the antigenic stimulus.

Practical Conclusions

Considering the results of the present investigations the following practical rules regarding prophylaxis against tetanus may be recommended.

An injury involving a risk of tetanus in a person not previously actively immunized should be treated with simultaneous active and passive immunization. Even better results would, no doubt, be achieved if the active immunization were not initiated until passive immunity had vanished. Still, since the attention of physician and patient is more easily directed to the prophylaxis against tetanus directly after an injury, simultaneous passive and active immunization is recommended. When, following a severe injury, a higher dose of serum than usual is required, the antigenic stimulus of the toxoid should be protracted so as to exceed the duration of the passive immunity. This protraction may be obtained by a second dose of toxoid or by postponing the first dose of toxoid.

In children prophylaxis against tetanus may be effected by the injection of a mixed tetanus and diphtheria toxoid. This procedure does not give rise to any disagreeable reactions and produces good results (Bigler and Werner, 1941). Any scheme of inoculation that affords good protection against diphtheria will also protect against tetanus arising from small injuries not treated by a physician. A booster dose should be given after major injuries, but serum prophylaxis should never be necessary if the child has once been actively immunized

Experience from the second world war has served to improve the prophylaxis in military forces during wartime. As a preparatory measure tetanus toxoid may be used in the military forces in peacetime and should be administered at the beginning and at the end of the first compulsory training period, the two injections being thus about one year apart. Booster doses should be given at mobilization and to all battle casualties. Under these circumstances serum should be unnecessary.

Summary

By determining the amount of antitoxin in the serum of patients after the generally adopted dose of 3,000 I.U. of tetanus antitoxin the minimum amount of circulating antitoxin wh ch gives protection against tetanus was estimated. The value found corresponds to 0.1 I.U. per ml. in a man weighing 65 kg.

This level may be achieved by an injection of adsorbed toxoid followed, at the time of injury, by a booster dose of the same toxoid. In man the main effect of the primary injection is not influenced by a simultaneous dose of 3,000 I.U. of antitoxin.

Conclusions are drawn as to the practical application of these experiences in prophylaxis against tetanus.

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THE VALUE OF LIQUOID FOR BLOOD CULTURE

BY

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No single medium has been devised that is suitable for the cultivation of every type of micro-organism from the blood. Penfold and others (1940) confirmed the findings of Elliott (1938) on the superiority of saponin broth for the cultivation of *Strep. viridans*, Hoare (1939) stressed the value of trypsin liver broth in the isolation of anaerobic streptococci from patients with puerperal infections, and more fastidious organisms need even more specialized media. Although these and other recommendations can be accepted, yet the difficulties of putting the various suggestions into practice have not been sufficiently emphasized. Butler (1937) in an important monograph enumerates four different media to be inoculated at the bedside. Penfold and others, while drawing attention to Butler's relatively elaborate technique, themselves recommend four media as a convenient range for routine work. Their methods suggest the need for considerable technical experience and indicate the care required to avoid contamination. The difficulty of maintaining sterility in technique at the patient's bedside is considerable, and is increased by each additional manipulation. In many quarters this has led to the belief that the bacteriologist must actually withdraw the blood as well as carry out the culture. This involves waste of time, even in a hospital, and is impracticable in a public service. Again it is frequently impossible for the bacteriologist to be present at the time most favourable for blood culture. Most of the difficulties arise from the need to distribute the blood into a variety of media immediately after withdrawal. If blood could reach the laboratory in a suitable state, with the infecting organisms unharmed, these troubles would be solved. It seemed likely from the published evidence that the use of liquoid would satisfy these requirements.

Liquoid (Hoffman–La Roche) is sodium polyanethol sulphonate. It has been recognized since 1930 as a powerful anti-coagulant (Demole and Reinert). Battistini (1932) first noted that its property of inactivating complement, thus destroying the bactericidal power of human plasma, made it valuable for blood cultures. Massa and Battistini (1934) used patient's blood treated with 0.16 per cent liquoid as a self-contained culture medium, and claimed excellent results in enteric fever, meningitis, pneumonia, and septic infections. Neisner and Volencová (1934) recommended "liquoid blood" as a transport medium, and many other Continental workers supported the original claim of its usefulness in investigating bacteraemic conditions. Most of these workers used liquoid in a concentration of 0.1 to 0.2 per cent. In this country von Haebler and Miles (1938) presented experimental evidence to show that liquoid in a strength of 0.05 per cent, either alone or incorporated in broth, was excellent for blood culture, but that higher concentrations were occasionally unfavourable. Hoare in general confirmed the findings of these workers but remarked that 0.08 per cent liquoid in liver broth was experimentally inferior to trypsin liver broth for the cultivation of anaerobic streptococci. Penfold and others used liquoid broth and "liquoid blood" parallel with other media in 160 routine blood cultures, and found no evidence to suggest any advantage from their use. Most of their cultures, however, were carried out with liquoid in a final concentration of 0.17 per cent, more than three times that recommended by von Haebler and Miles.

Further information on the rationale of the use of liquoid was provided by Auxilia (1934), who pointed out that, in addition to destroying bactericidal complement, it interfered with the phagocytic activity of leucocytes. Allgöwer (1947) went further, and showed that in experimental tissue cultures traces of liquoid completely prevented phagocytosis, and concentrations as low as 0.01 per cent exerted an obvious inhibitory effect on leucocytic migration.

Accordingly it was decided to use liquoid-treated blood both as a transport medium and as a culture medium and to carry out routine aerobic and anaerobic cultures with small volumes of the blood on various media in addition to incubating it as a self-contained culture. At the same time the results of parallel cultures in an average "enriched" broth medium were to be compared.

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Methods

These were selected as suitable for the large general hospital in which this work was carried out.

Blood-culture outfit.—This consisted of a 10-oz. bottle with a perforated cap and a rubber liner, containing 100 ml. papain digest broth and 5 ml. of sheep's blood (boiled blood broth). To this was attached by a string loop a small 1-oz. (H. 53 U.G.B.) bottle with the screw cap similarly adapted containing 1 ml. of 0.3 per cent liquoid in saline. Both bottles were sterilized by autoclaving. A dry sterilized syringe and two needles were also provided.

Ward technique.—This varied according to the preference of different hospital units. The method recommended was to swab the patient's arm first with spirit-soap or ether-soap, then with surgical spirit. Approximately 10 ml. of blood was taken from the patient and the needle detached from the syringe. The second needle was then fitted to the syringe with a sterile forceps, and 5 ml. of blood was ejected into each bottle by perforating the rubber cap.

Laboratory technique.—Both bottles were incubated for 24 hours except under special circumstances; 0.5 ml. of "liquoid blood" was then distributed by pipette to each of two tubes, one of nutrient broth, one of thioglycollate broth (Brewer, 1940). At the same time a drop of blood was spread on a blood-agar plate and another was smeared on a slide. From the ordinary blood culture bottle subcultures were made to blood agar in the usual way. This routine was repeated after a further 24 hours' incubation of the original bottles. Anaerobic plate cultures were carried out as required to confirm the character or establish the identity of organisms seen in films from the boiled blood broth or Brewer's medium but which failed to grow aerobically on solid media. The subcultures and original blood cultures were examined regularly and were not considered negative until after 7 days' incubation. For special examinations the "liquoid blood" was occasionally subcultured to other media, according to indication.

Results

Eight hundred and sixteen blood cultures were made, of which 170, from 141 patients, were positive. The Table shows the relative values of the methods used. The times given indicate approximately the period of incubation before subculture to a solid medium was successful. In most instances the "liquoid blood" inoculum was taken directly from the blood culture bottle, but it was occasionally taken from an early subculture in nutrient or thioglycollate broth.

In the course of this work liquoid was found to have advantages other than the maintenance of blood in a state suitable for diagnostic culture. Sometimes measured amounts of "liquoid blood" were dispersed in poured plates for the estimation of degrees of bacteraemia, and the blood plasma obtained on standing or by centrifuging was also sometimes used for agglutination tests. Repeated examinations showed no significant difference between the agglutinin titres of serum and "liquoid plasma" from the same source. Thus it was occasionally possible to obtain a serological diagnosis by demonstrating a higher titre in a serum taken for Widal examination than in the "liquoid plasma" of an earlier blood culture. On one occasion a "liquoid blood" culture was obtained from a nurse who had been ill for one week with fever and vague pains in the joints. Four days later her serum Widal test was positive to *S. paratyphi-B* 1/5,000 (H), 1/320 (O), while the titre of the plasma from her earlier blood culture was only 1/640 (H), negative (O). From repeated examinations of similar specimens during periods of incubation the loss in agglutinin titre during such incubation was known to be trifling, and the rise in titre so demonstrated established the diagnosis of paratyphoid fever. It was also possible to obtain an early indication of enteric infection by serological tests on plasma from a "liquoid blood" culture and later to confirm the diagnosis by the isolation of the infecting organism from the same specimen. *S. paratyphi-B* was isolated from such a blood culture with a specific plasma titre of 1/3,200 (H), 1/50 (O). By similar methods it was occasionally possible to determine the aetiological importance of organisms of doubtful pathogenicity. Thus the recovery of *B. coli* from a patient's blood became significant when the "liquoid plasma" was found to agglutinate the organism to a titre of 1/1,280. On three occasions the diagnosis of Weil's disease was suggested by a positive serological test on liquoid plasma before it was clinically suspected.

Discussion

This is the largest series so far reported of blood cultures in which the use of liquoid has been compared directly with more usual methods. "Liquoid blood" has proved to be a good culture medium, but it is now suggested that liquoid is most valuable as a vehicle in which a specimen of blood can be brought to the laboratory for the bacteriologist's investigations. It can then be distributed into various culture media as considered advisable. This answers any criticism suggested by the results of Hoare or of Penfold and others, who used "liquoid blood" itself as a culture medium. Direct trial against a variety of

other recommended culture media has not been attempted, but subculture to digest broth, Brewer's thioglycollate broth, and blood agar appears to allow successful isolation of most of the bacteria likely to be encountered in hospital practice. Penfold and others, using saponin broth, obtained eleven positive results in eleven cultures from patients with *Strep. viridans* infections, figures much superior to those obtained with other culture media used in parallel. In this series forty-six positive *Strep. viridans* cultures out of a possible total of forty-eight were obtained both from "liquoid blood" and from the enriched broth used in parallel. That the failures, one by each method, were probably due to the chance distribution of bacteria in blood specimens, is suggested by the occasional finding that in one blood culture the infecting organism might grow luxuriantly from boiled blood broth and poorly, or not at all, from liquoid, while in a further specimen from the same individual a few days later the reverse would be experienced. During this period of trial no case of subacute bacterial endocarditis due to this infection came to necropsy without a previous diagnosis by blood culture; and it is unlikely that any established *Strep. viridans* septicaemia remained undetected. It can therefore be assumed that for ordinary aerobic bacteria the use of "liquoid blood" for transport and subculture into the suggested media is as good as the use of other recommended media, but for certain bacteria it has advantages. In this series anaerobic streptococci, fusiform bacilli, and *F. necrophorus* were isolated more frequently from the "liquoid blood" specimen. This was undoubtedly due to the routine use of a convenient clear anaerobic fluid medium (Brewer's) for subculture. Much heavier inocula can be used than in subcultures from ordinary broth to solid media for anaerobic incubation, with the consequent greater opportunity of recovering such bacteria. Routine anaerobic incubation of subcultures to solid media is also thought in many busy laboratories to be unduly laborious. Early growth in this thioglycollate medium is much more easily seen than in Robertson's meat medium or in liver broth recommended by other workers. Again, an organism such as *L. icterohaemorrhagiae* can never be grown from any ordinary culture medium, yet a small amount of a "liquoid blood" specimen can readily be subcultured to a special leptospiral medium if suspicion of such an infection arises. Although leptospirae were recovered only on one occasion in this series, the general suitability of the procedure has been demonstrated by numerous isolations from the blood of infected guinea-pigs (Stuart, 1943). Since this investigation was completed Allison (1944) has also mentioned the use of liquoid as a vehicle for the transport of blood culture specimens.

TABLE

COMPARATIVE CULTURE RESULTS FROM BOILED BLOOD BROTH AND "LIQUOID BLOOD"

Organism	No. of patients	Total no. of positive cultures	Boiled blood broth				Liquoid				+ B − L	− B + L
			24 hrs.	48 hrs.	Later	Total	24 hrs.	48 hrs.	Later	Total		
Staph. pyogenes ..	38	42	22	12	3	37	25	11	2	38	4	5
Strep. pyogenes ..	20	21	19	—	—	19	18	1	0	19	2	2
Strep. viridans ..	37	48	23	20	3	46	27	16	3	46	2	2
Strep. non-haemolytic ..	4	5	2	0	1	3	2	1	1	4	1	2
Strep. anaerobic ..	3	3	0	0	2	2	0	2	1	3	0	1
Strep. pneumoniae ..	9	9	8	0	0	8	9	0	0	9	0	1
Micrococcus Sp. ..	1	4	2	2	0	4	1	2	1	4	0	0
N. meningitidis ..	1	3	2	1	0	3	2	1	0	3	0	0
H. para-influenzae ..	2	3	0	1	2	3	0	1	2	3	0	0
S. typhi	2	2	0	1	1	2	0	1	0	1	1	0
S. paratyphi-B ..	13	16	8	5	2	15	4	6	5	15	1	1
S. enteritidis	1	2	1	0	0	1	0	1	0	1	1	1
B. coli	5	6	6	0	0	6	6	0	0	6	0	0
B. proteus	1	1	1	0	0	1	1	0	0	1	0	0
F. fusiformis	1	1	0	0	0	0	0	0	1	1	0	1
F. necrophorus ..	2	3	0	1	1	2	0	2	1	3	0	1
L. icterohaemorrhagiae	1	1	0	0	0	0	0	0	1	1	0	1
Total ..	141	170	94	43	15	152	95	45	18	158	12	18

+ B − L = Positive from broth, negative from liquoid.
− B + L = Negative from broth, positive from liquoid.

Attention has been drawn for the first time to the use of "liquoid plasma" for agglutination tests. This allows the same specimen to be used for a Widal test as well as for culture for any likely organism. The possibility of making a serological diagnosis by the detection of a rising specific titre by retrospective examination of the plasma from an earlier blood culture is also valuable on occasion.

In general medical practice away from hospital facilities the difficulty of obtaining sterile syringes, etc., greatly reduces the practicability of using blood cultures for diagnosis. Yet the general practitioner often sees a patient at a time when blood culture is a vital diagnostic procedure. In this sphere the use of a vacuum venule is especially suitable, especially if it contains liquoid instead of the culture media more commonly employed. Some years ago a venule was marketed containing 1 ml. of 1 per cent liquoid. This was much too strong, and more recently a venule containing 1 ml. of 0.3 per cent liquoid has been introduced, which will produce a final concentration of 0.05 per cent in the average volume of 5 ml. of blood collected. This concentration has been used in the present series of cases and has proved entirely satisfactory.

Liquoid has been found useful by Fleming (1943) in determining the bactericidal power of blood after penicillin therapy, but it has been shown by May, Voureka, and Fleming (1947) to be unsuitable for the titration of streptomycin in body fluids. Its capacity to interfere with streptomycin activity may, however, prove useful in the isolation of infecting bacteria from patients under treatment with this antibiotic agent.

Summary

Liquoid (Hoffman–La Roche), in a final concentration of 0.05 per cent, has been used in blood cultures, for making a self-contained culture medium, and as a transport vehicle. The results in 816 blood cultures have been compared with those from a boiled blood-broth medium used in parallel. Of a total of 170 positive cultures, 157 were obtained from "liquoid blood," and 152 from the enriched broth medium. In a few instances fastidious bacteria were cultured with greater facility from the "liquoid blood," but its chief advantage appears to be the simplicity of its use. The taking of a single sample of a patient's blood into one container minimizes the risk of contamination, and the benefit of using a variety of culture media can be readily obtained by appropriate subcultures in the laboratory. Thus the chief value of liquoid is in transporting blood specimens to the laboratory in a suitable state for bacteriological investigation.

An unexpected advantage from the use of liquoid in keeping the blood fluid has been that the plasma can be used for agglutination tests. In the series described, "liquoid plasma" has proved suitable for Widal tests and for leptospiral and other agglutination tests. The destruction of complement by liquoid, of course, renders specimens unfit for complement fixation reactions.

A suitable concentration of liquoid in vacuum venules provides the simplest and most effective means in general practice of obtaining blood for culture.

This work was carried out between 1938 and 1943 in the Bacteriology Department of the Royal Infirmary, Glasgow, to which due acknowledgment is hereby made. Messrs. Hoffman–La Roche are thanked for supplying 3 g. of liquoid and for providing several references not readily available.

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THE USE OF THE SELECTIVE INHIBITORY ACTION OF TOLUENE ON COLIFORM BACILLI IN ROUTINE CULTURES

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Benians (1913) demonstrated that all Gram-negative organisms were destroyed by toluene, which had an inhibitory action on diphtheroid bacilli and streptococci but little effect on spores and staphylococci.

Webster (1942) suggested the use of toluene to suppress Gram-negative bacilli in mixed broth cultures; he added 10 per cent of toluene to broth cultures, shook them at intervals, and allowed them to stand for five minutes, by which time the toluene had risen to the surface. He removed a drop of the treated culture, taking precautions to avoid removing any toluene. Using this technique we have obtained results too variable to be of value, as sometimes coliform bacilli would survive or streptococci succumb. "Occasional" shaking is too haphazard, but vigorous shaking gives consistent results so long as the temperature of the broth is controlled.

Demonstration of the Effect of Temperature on the Action of Toluene

A set of bijou bottles, each containing 2 ml. measured from the same overnight broth culture of the organisms shown in the Table, were kept at each of the following temperatures: 4°, 23°, and 37° C. One of the bottles from each set was used as a control. A standard drop of the bacterial suspension was inoculated and spread evenly on to a MacConkey plate when coliform bacilli were being tested, or a blood plate for the other organisms; after overnight incubation a colony count was made. Similar counts were made after exposure of the organism to the action of toluene for times varying from 5 seconds to 3 minutes.

These results are summarized in the Table, which shows that *B. proteus*, *Ps. pyocyanea*, and the coliform bacilli generally were either suppressed by contact for 5 seconds at 37° C., or were reduced from hundreds of colonies per drop to about 7 or 10 in the same volume. At lower temperatures these Gram-negative organisms survived for much longer periods, so that at 4° C. they were present in large numbers after 2 minutes' exposure to toluene. The Gram-positive organisms withstood 2 minutes' exposure to toluene at 37° C., though there was a slight reduction in the number surviving after this time. Experiments with *Staph. aureus* and *Str. pyogenes* are duplicated to show the effect of toluene on small numbers of these organisms.

Organisms Tested and Shown to have Resistance to Toluene

Many strains of *Staph. albus*, *Staph. aureus*, α, β, and γ haemolytic streptococci, pneumococci, and diphtheroid bacilli encountered in routine work were tested and found to be resistant to toluene. As few strains of the following organisms were encountered, stock cultures were tested and were resistant: anaerobic streptococci 1 strain, *Cl. welchii* 6 strains, *Cl. tertius* 1 strain, *Cl. oedematiens* 1 strain.

The technique has now been employed for a period of over three years on routine cultures which included wound swabs, faeces, urines, and a few sputa, vaginal and cervical swabs, and throat swabs which have contained coliform bacilli: over five hundred strains of *B. proteus*

TABLE

REPRESENTATIVE RESULTS SHOWING THE EFFECT OF TEMPERATURE ON THE LETHAL ACTION OF TOLUENE

Organisms	Temp. °C.	Control	Time of exposure to toluene					
			5 secs.	15 secs.	30 secs.	1 min.	2 mins.	3 mins.
Ps. pyocyanea	4	+++	+++	+++	++	++	0	0
	23	+++	+++	++	+	12	0	0
	37	+++	2	—	—	—	0	0
B. proteus	4	+++	+++	++	+++	+++	+++	++
	23	+++	48	30	—	—	—	—
	37	+++	7	—	—	—	—	—
Diphtheroid bacilli	37	+++	+++	+++	+++	+++	++	0
Staph. aureus	37	+++	+++	+++	+++	+++	+++	+++
	37	7	0	5	6	9	0	0
Ana robic indifferent streptococci ..	37	+++	+++	+++	+	43	0	0
Str. pyogenes	37	+++	+++	+++	+++	+++	++	0
	37	25	0	37	42	40	0	0

+++ = About 500 to 800 colonies. ++ = 200 to 500 colonies. + = 50 to 200 colonies.
48, 42, 40, etc. = Number of colonies grown. — = Sterile. 0 = Not examined.

and three hundred strains of *Ps. pyocyaneus* have been encountered, and a few of these failed to be killed in our standard time of exposure to toluene, but when we obtained a fresh specimen the coliform bacilli were inhibited. Two insensitive strains of each organism were subcultured and each continued to be insensitive, but fresh specimens from the cases concerned contained *B. proteus* or *Ps. pyocyaneus* which were fully sensitive to toluene; if possible, therefore, when one of these organisms appears to be insensitive a fresh specimen is obtained as the insensitive variants may not be present.

Comparison of the Toluene Technique with the "Fry" (1932) Plate

Sixty-seven specimens containing a mixture of coliform bacilli and Gram-positive organisms were examined by both methods. The toluene technique was simpler and less time-consuming; the colonies were directly visible and colonial characteristics were unaltered, giving ease of identification and subculture. On the "Fry" plate, colonies—other than haemolytic streptococci—had to be subcultured for identification by pricking through the agar layer, a relatively difficult technique.

The Routine Plate for Wound Swabs

This is a blood agar plate that has a central ditch cut out and removed; the ditch prevents *B. proteus* swarming across to the toluene-treated side of the plate. Over the outer segment of each half-plate gentian violet solution is spread (Fleming, 1942). As each batch of gentian violet varies in its inhibiting power, the optimum concentration must be established experimentally; our sample needed a dilution of 1/30,000. The gentian violet solution is made up in sterile industrial spirit (Robson, 1945), as it is found that the plates then dry much more quickly than with an aqueous solution of the dye. The plate is dried before planting as there is a tendency for the cultures to become confluent if the gentian violet is spread after inoculation.

1. The swab is first planted on one half of the plate described above and a smear made for a Gram stain.

2. The swab is emulsified in 2 to 3 ml. of broth, in a bijou screw-capped bottle; the bijou bottles are kept ready for use at 37° C.: if there is likely to be an interval before the toluene is added, the bijou bottle is replaced in the incubator to prevent cooling.

3. Toluene, approximately one-tenth of the volume of the broth, is added to the emulsion.

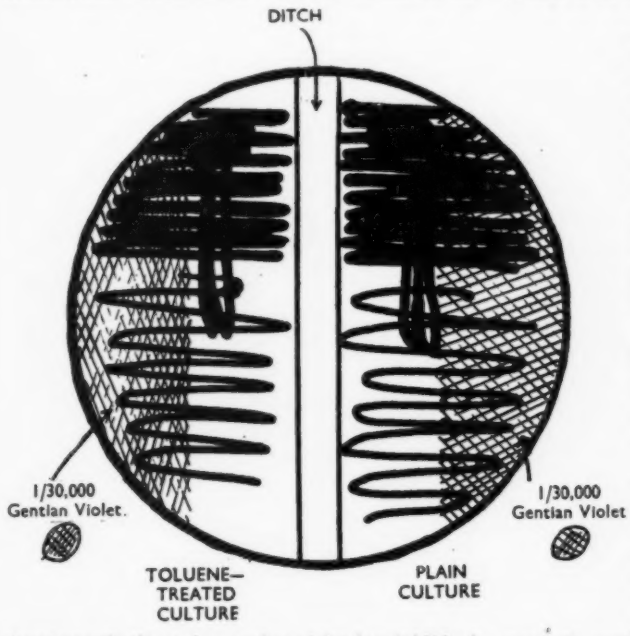
4. The bottle is recapped and immediately shaken vigorously for 15 to 20 seconds.

5. With a sterile Pasteur pipette one drop of the suspension is placed on the second half of the blood plate and is then spread in the routine manner; we do not wait for the toluene to rise and settle out before we remove the broth for planting.

This one plate allows the easy recognition of the relative numbers of the different organisms present. Any coliform bacilli present will grow on the side of the plate planted without treatment by toluene; if few coliform bacilli are present

they may be swamped by a heavy growth of staphylococci, whereas the use of gentian violet allows them to appear as discrete, recognizable colonies.

The toluene-treated culture will contain no Gram-negative organisms and the relative numbers of Gram-negative and positive organisms will be shown on the two halves of the plate. The part of the plate untreated by gentian violet will show the staphylococci and diphtheroid bacilli present, and alongside this area will be shown the streptococci where the gentian violet has inhibited the staphylococci and diphtheroid bacilli (see Figure).



Faeces.—After the specimen has been planted on the usual special media for Salmonella-dysentery groups, an emulsion is made in broth and this is treated with toluene in the same fashion as a wound swab emulsion ; occasionally the coliform bacilli fail to be killed by exposure to toluene for 15 seconds and this may be explained by the work of Benians (1913), who showed that fatty and albuminous substances inhibit the action of toluene.

Urines.—The centrifuged deposit is planted in the laboratory's usual manner, and then some of the deposit is added to the warm broth and treated with toluene as previously described.

Discussion

Many techniques have been evolved to suppress coliform bacilli generally and *B. proteus* in particular, as their tendency to overgrow other organisms obscures the complete bacteriological picture in mixed infections. Inhibiting agents (phenol, alizarin red, etc.) can be incorporated in the medium, and Bray (1945) exposes the planted plate to ether vapour, but these methods necessitate the use of duplicate plates if the complete picture is to be given.

The 8 per cent agar plate, Hayward and Miles (1943) provides colonies unlike those on ordinary laboratory media, and therefore suffers from the same drawbacks as the " Fry " plate, although the latter was introduced primarily for the isolation of haemolytic streptococci in the presence of *B. proteus*. Beattie's (1945) method only inhibits the swarming of *B. proteus*, so if many coliform bacilli are present they may hide small numbers of Gram-positive organisms.

Toluene appears to inhibit the growth of all pathogenic organisms if it is left long enough in contact with them, the inhibiting action being accelerated by increased temperature. The Gram-negative organisms are very susceptible to its action, whereas the Gram-positive organisms are relatively resistant. We now employ toluene in the primary cultivation of any specimen that is likely to contain coliform bacilli ; we use it on all wound swabs and faeces, and in some cervical, urethral, and aural swabs, urines, post-mortem material, and sputa.

Summary

A method of inhibiting coliform bacilli in cultures is described ; also a new method of applying gentian violet in a composite plate which gives a complete and unobscured bacteriological picture on the one culture.

We would like to thank Dr. L. Colebrook and Dr. W. H. McMenemey for their advice and help.

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TECHNICAL METHODS

A MACHINE FOR WASHING WASSERMANN TUBES

BY

E. A. ATKINSON

One of the most irksome tasks in a pathological department is the washing of test-tubes. Particularly is this so when a thousand or more tubes have to be washed at the end of a long day doing Wassermann and Kahn tests. If the task is postponed to the next day the cleaning process becomes more difficult. It seemed that a machine was needed for the mechanical rinsing of tubes immediately after the conclusion of the tests. The apparatus to be described has been in use in this laboratory for several months and serves as an efficient rinsing machine.

Description of Apparatus

Test-tubes are contained in revolving circular racks sprayed from below by a system of water jets. The racks are 7 in. in diameter and have bottoms of 18-gauge copper wire mesh, six to the inch. Each rack is divided into four compartments by partitions radiating from a central hollow shaft. The jets are thirty or so in number, and so arranged as to spray a sector of the bottom of the rack immediately above. One row of jets is inclined at an angle—this provides a lateral thrust and maintains a slow revolution of the rack.

Method of Use

A rack is filled with tubes placed upside down (280 ¼-in. tubes or 160 ½-in. tubes) and the glass cover (an inverted mouse jar) is placed in position. The tubes are sufficiently rinsed after the water has run for two or three minutes, when another full rack is placed in position and the process is repeated. It is advisable to have at least half a dozen racks. The racks are partly drained and then placed in the hot air oven. Given a sufficient number of racks, the tubes may be left in position until required again.

The method is not suitable for very dirty or greasy tubes requiring drastic chemical treatment, but is suitable for large-scale mechanical rinsing. In my experience the tubes emerge bright and clean from the drying oven in spite of a very hard water supply.

The machine illustrated was made by Messrs. Jackson, of 348, City Road, London, E.C.1.

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A MACHINE TO ASSIST IN RHESUS-GROUPING

BY

E. A. ATKINSON

The routine examination of blood during the antenatal period for the rhesus factor has become an important function of many hospital laboratories. The area served by this department provides more than 10,000 such specimens a year, and it was important to find a method of performing the agglutination test with anti-D serum involving as little effort or chance of confusion as possible. The device to be described employs the tube test, and the reading of the results is simplified by the projection of a small lantern image of the red cell sediment on a screen.

Description of Apparatus

The machine consists of a circular rack revolving round a vertical axle. Above is suspended a dark-ground illuminator: below is fixed a small lantern projection lens. The rack consists of two circles of one-eighth gauge steel plate (13 in. diameter) drilled to take two concentric rings of ½-in. × 3-in. tubes with a bottom plate of ¼-in. plate glass. All three are joined to a hollow-threaded central shaft. The compound projection lens is made of two biconvex lenses, each 3 cm. diameter and of 6 cm. focal length and placed directly under the outer ring of tubes. The holes are numbered to take 32 tests.

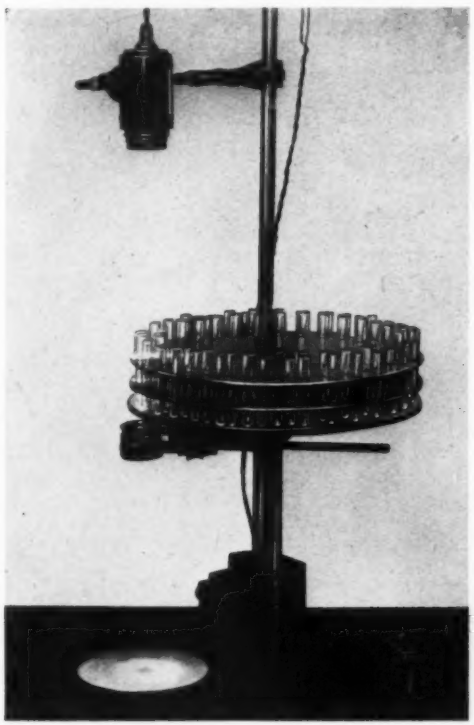

FIG. 1.—The rack.

Method of Use

The inner row of tubes is used for the preparation of the washed red cell suspensions. The outer circle of holes is then loaded with the reaction tubes. One large drop of red-cell suspension is then transferred from each inner tube to the corresponding reaction tube. A very small drop of anti-D serum is then added to each reaction tube and the whole rack is placed in the incubator. After periods of one and two hours the rack is placed on the axle and readings are taken. In actual practice the positive results are usually clearly readable after about half an hour.

Advantages Claimed

The machine is useful for the mass testing of routine specimens. As with the Chown method, rapid settling out of agglutinated particles occurs, but with rather less economy of serum. At the same time, the advantages of a tube test are retained. By the device described, the reading of the result of the tube test is simplified, and the lantern images correspond well with the microscopic findings.

I am grateful to the hospital engineer, Mr. F. G. Bradley, and his staff, who constructed the rack.

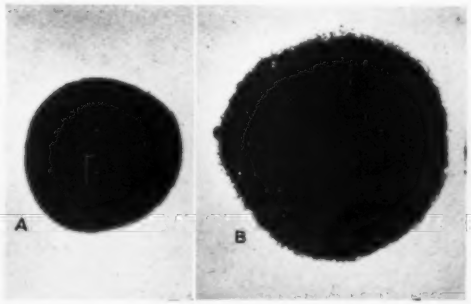

FIG. 2.—(*a*) Negative result. (*b*) Positive result after one hour's incubation.

OBITUARY

GORDON WILKINSON GOODHART

Dr. G. W. Goodhart, M.D., F.R.C.P., Bunny Goodhart as most of his friends liked to think of him, died in London on July 16 just before his 66th birthday. He was the younger son of Sir James Goodhart, a consulting physician in the great Victorian tradition. He was educated at Westminster School, Trinity College, Cambridge, and Guy's Hospital, graduating M.B. in 1908. At Guy's he became successively house-physician, Gull research student, Douglas demonstrator in pathology, and senior assistant bacteriologist. Subsequently he worked in Berlin, and for a time with Aschoff at Freiburg, returning to become clinical pathologist at University College Hospital. In the 1914–18 war he was in France with the R.A.M.C., and served as pathologist to the Second London General Hospital. In the following years at University College Hospital, apart from teaching students and his own junior staff, he carried on continuous research with Charles Bolton on gastric secretion and constantly helped and stimulated the fundamental work of Price Jones on red cell size. In 1931 he was appointed as one of the first four pathologists to the London County Council, and he did much to establish and develop their highly successful service. He worked first at the Archway Group Laboratory and then at St. Mary Abbots. He was in due course President of the Section of Pathology of the Royal Society of Medicine, of the Medical Society of London, and of the Association of Clinical Pathologists. To this Association in its early years he gave unfailing help and encouragement.

Physicians and surgeons were always to be found in Goodhart's laboratory at University College Hospital, or later in his London County Council laboratories, discussing their problems with him. He himself was constantly to be seen in the wards looking at the patients. The knowledge he gained in his laboratory only became real to him in relation to the individual patient. Case reports or pathological reports alone were meaningless and both must be interpreted in relation to the actual human being. So he taught a succession of students, assistants, and colleagues, for from his early days at U.C.H. all medical students served a three months' clerkship in his department. This, though now an accepted procedure in medical training, was an innovation, part of Goodhart's contribution to the medicine of his time. Many of his pupils practising in very different fields to-day when they meet agree that Bunny Goodhart taught them what really mattered in their medicine. His original contributions to knowledge were all sound, but so much of what he had learnt by careful observation

GORDON WILKINSON GOODHART

and experience he never wrote down but handed on in his day-to-day teaching and conversation. This is especially true in the expanding field of haematology.

During the last five years he was often very ill, but he carried on with a courage and gaiety that will always be an inspiration to remember. His hospital might quite literally be brought down on top of him, but there he still was ready to help both with medical and personal problems. Still finishing the last story, he saw one out over the heaps of rubble just as in safer days he had seen one down the laboratory stair. Those long stories were very much part of the man: they were never unkind, they were often shrewd, and he told them with such evident enjoyment himself that the listener enjoyed them too.

Gordon Goodhart had a fine mind but no great ambition to achieve academic or worldly distinction. He was content to do his day's work well, to return after tea to an exceedingly happy home with his wife and their three children, to keep alive a host of friendships; but when the medical history of the early twentieth century comes to be written he will be remembered as one of the leaders of a small group of men who, because of their own skill as physicians combined with their love and understanding of the more precise discipline of the laboratory, created that hybrid despised by some but none the less essential to the developing medical sciences—the clinical pathologist. His friends to-day miss him and will remember him as, in the fullest sense of the words, "a beloved physician."

JANET VAUGHAN.

REVIEWS

Pathologische Anatomie und Chemotherapie der Infektionskrankheiten. By Gerhard Domagk. Stuttgart: Georg Thieme Verlag. 1948. Pp. 424. 133 illustrations. Price, bound, D.M. 48.

Hardly more than a generation has passed since British medicine looked to Germany for inspiration and for advanced books, perhaps in no branch of medical science more than in the field of pathology. English textbooks are now as eagerly sought by medical students in most parts of the world. Nevertheless a new publication in German still evokes the interest of British students and research workers, especially one from the pen of Professor Domagk, whose name is so closely associated with pioneering research on the sulpha drugs. The general plan of relating the action of the antibacterial drugs to the pathological process of infection with a detailed study of the tissue changes is a good one and is well done. The student will also benefit from the author's classification of infections from a pathogenetic point of view, although this classification should not be regarded as rigid. The disturbing feature of the book is that, although the title suggests a wider field, in fact only bacterial diseases are dealt with and even they are not fully covered. The reader will progressively come to realize that the term "infectious disease" is here used almost synonymously with those infections which are affected by the sulpha drugs. The book is, in fact, a survey of sulphonamides and covers a wide field of research into their uses and actions: as such it is an excellent reference book. The author may naturally have stressed his own particular interests, but the need for full information on all modern antibiotics and their uses and limitations is urgent, particularly for German doctors who have had till now such slight experience of penicillin, streptomycin, etc., and who may be given a one-sided impression by this book. It is to be hoped that this deficiency will be remedied in the next edition.

H. G. KOHLER.

British Drug Houses have produced a pamphlet entitled "Introductory Notes on Chromatography," which they are prepared to send without charge to clinical pathologists who may be interested in this subject. The monograph is clearly meant to cover as wide a range as possible. Those interested in the specific aspect of the field will find the bibliography useful.

NICHOLAS MARTIN.

J. clin. Path. (1948), **1**, 322.

ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.*

BACTERIOLOGY

The Recognition of Toxicogenic Bacterial Strains *in vitro*. ELEK, S. D. (1948). *Brit. med. J.*, **1**, 493.

This *in vitro* test for the detection of toxicogenic strains of various organisms is based on the principle of flocculation of a toxin-antitoxin mixture. In order to establish optimum conditions for the latter, the author pours plates and embeds, while the medium is still soft, a strip of filter paper previously dipped into specific antitoxin. After the medium solidifies the plates are inoculated by streaks at right angles to the filter paper. The toxin produced by the subcultures and the antitoxin contained in the filter paper diffuse into the medium, and after 24 to 48 hours precipitation occurs in a line and at an angle of about 45 degrees at either side of the inoculum, thus appearing as an arrow-head. Technical details are given and non-specific reactions are discussed. The method appears to be particularly suitable for the detection of virulent strains of *Corynebacterium diphtheriae* and, in the hands of the author, yielded results identical with those of the guinea-pig test.

R. Salm.

Post-examination of BCG-material. (In English.) TÖRNELL, E. (1947). *Acta tuberc. scand.*, **21**, 241.

The author reviews the results of BCG vaccination as carried out at, or in connexion with, the Dispensary in Borås, Sweden, run by the Västerås Sanatorium. Between 1935 and 1945 over 10,000 persons were vaccinated, including child and adult contacts, school-leavers, factory-workers, and army conscripts. From 1946 onwards all newborn babies in the maternity homes were also vaccinated. Technique and isolation precautions are described in detail. The dose of vaccine used has gradually been increased over the years from 0.05 mg. to 0.06 to 0.08 mg. Wallgren's intracutaneous method was employed, and a weal 10 to 12 mm. in diameter was aimed at.

Out of 10,963 individuals only 17 developed tuberculosis later; 12 of these, who had been exposed to infection before vaccination was completed, fell ill within 3 years of vaccination; but the other 5 became ill after 4 years. Full and careful follow-up and records of case histories are given, with 119 unvaccinated tuberculin-negative relatives acting as controls.

Allergy and immunity do not always run parallel. Although allergy fades out in about 4 years, a changed level of reaction does persist, indicating a certain degree of immunity. Nevertheless, all vaccinated persons who are tuberculin-negative after 4 years should be revaccinated and persons who react only weakly should be retested each year.

***p*-Aminosalicylic Acid in the Chemotherapy of Tuberculosis.** (*p*-Aminosalicylsäure in der Chemotherapie der Tuberkulose.) RAGAZ, L. (1948). *Schweiz. med. Wschr.*, **78**, 332.

There was rapid improvement in 60 to 70% of pulmonary tuberculosis, with amelioration of all the clinical signs, when treated with 10 to 15 gr. of *p*-aminosalicylic acid daily on alternative weeks. A 10% solution of the drug may be employed in the local treatment of tuberculous empyema and abscesses. It has so far failed to cure patients with tuberculous meningitis and miliary tuberculoses, but clinical experience is still too small to permit critical assessment of its value.

Streptomycin Resistant Tubercle Bacilli. Their Development during Streptomycin Therapy of Pulmonary Tuberculosis. FISHER, M. W. (1948). *Amer. Rev. Tuberc.*, **57**, 53.

Sensitivity of Tubercle Bacilli to Streptomycin. An *in vitro* Study of some Factors Affecting Results in Various Test Media. FISHER, M. W. (1948). *Amer. Rev. Tuberc.*, **57**, 58.

The sensitivity to streptomycin of tubercle bacilli from the sputa of 20 patients suffering from pulmonary tuberculosis, and receiving 1.8 g. streptomycin daily for 120 days, was studied, the Dubos albumin-"tween 80" medium being used. In 15 patients the bacilli remained sensitive. In the remainder sensitivity was retained for up to 11 weeks, after which varying degrees of resistance developed. Since this finding contrasted with that of Youmans *et al.*, who found that resistance developed in 8 out of 12 patients, strains isolated at the end of therapy from the 20 patients of the present series were compared by sensitivity tests in Dubos medium and in Youmans' medium (which contains 10% human plasma and 2% glycerin). Eleven of the 20 strains were resistant in the Youmans' medium (growing in 10 μg. or more of streptomycin per ml.).

The factor responsible for these discrepancies was found to be the "tween 80" (oleic acid ester). In the Dubos basal medium alone (without albumin or tween) all these strains were resistant to 1,000 μg. streptomycin per ml. Addition of albumin made no difference, but if

tween 80 was added (without albumin) the strains were sensitive to 1 μg. per ml.—that is, the effect of streptomycin was enhanced 1,000-fold. When albumin as well as tween was added the sensitivity was still increased but not as fully as when tween 80 was used alone. The potentiation of streptomycin by tween 80 may be due to surface-activity causing easier absorption of the antibiotic.

Streptomycin Treatment in Intestinal Tuberculosis. (Die Streptomycinbehandlung der Darmtuberkulose.) MARKOFF, N. (1948). *Schweiz. med. Wschr.*, **78**, 329.

A preliminary report is given of the beneficial effect of streptomycin in the treatment of 5 cases of ulcerative intestinal tuberculosis. The streptomycin was administered parenterally and by means of high enemata, in a strength of 250 mg. per 500 ml. of normal saline. Daily enemata are given for 14 to 15 days and are well tolerated. By elevating the pelvis and placing the patient on the right side it is possible to make the solution pass through the ileocaecal valve. A 6-month survey showed that there was invariably an improvement in the radiological findings, a decrease in the toxaemia, and a general subjective improvement. Complete regression of the intestinal lesions has not yet been observed. The results so far are, however, eminently satisfactory, considering the hopelessness of the condition. Streptomycin in intestinal tuberculosis must, however, be given in a hospital or sanatorium where the pulmonary and general condition can be accurately assessed. *Harold Jarvis.*

Recovery of Streptomycin from Urine. MILLER, J., and ROWLEY, D. (1948). *Lancet*, **1**, 404.

In the treatment of tuberculous patients with streptomycin it was found that about 50% of the drug was excreted in the urine at a concentration of about 1,000 units per ml. The streptomycin could be recovered from the urine, by simple adsorption methods, in a sufficiently pure form for reinjection, thus effecting a saving of the drug during the present shortage. *R. Wien.*

Streptomycin in the Treatment of Tularemia. BENSON, R. C., and HARWELL, A. B. (1948). *Amer. J. med. Sci.*, **215**, 243.

The authors summarize the clinical data in 56 cases of tularemia treated by streptomycin; 15 were examples of pleuro-pulmonary tularemia, in 10 of which the primary site of infection appeared to be the lung or pleura. The authors point out that to be beneficial streptomycin must be administered before the twelfth day of illness, otherwise it will not prevent the breaking down of infected lymph nodes. Streptomycin therapy was highly efficacious in modifying the course of the disease in every case, being most effective in the severest cases. Duration of treatment varied from 4 to 18 days, averaging 9.1 days. The total dosage was from 1.9 to 20 g., averaging 8.1 g. Only 3 patients manifested any intolerance, but in none was it necessary to discontinue treatment.

Jos. B. Ellison.

Streptomycin in Surgical Infections. IV. Peritonitis. PULASKI, E. J., SEELEY, S. F., and MATTHEWS, C. S. (1947). *Surgery*, **22**, 889.

A clinical study was carried out on young men who were in good health before the onset of peritonitis. In addition to streptomycin the majority of the patients had full supportive therapy. The authors conclude that streptomycin has a place in the treatment of peritonitis, results of therapy by this agent alone running approximately parallel to those with large doses of penicillin. Streptomycin appeared to have little effect in localized infections but was useful in a spreading peritonitis, effecting localization in such cases. Used with penicillin, it was effective in many cases which failed to respond to penicillin with or without sulphonamides.

A. E. Porritt.

Streptomycin in Human Plague. KARAMCHANDI, P. V., and SUNDAR RAO, K. (1948). *Lancet*, **1**, 22.

Five moribund patients, all proved by gland puncture to be suffering from plague during an epidemic in the Madras area, made good recoveries. Improvement started on the second day after about 1.5 g. of streptomycin had been given; 4 g. was a sufficient total dose when given 3-hourly in intramuscular injections of 0.125 g. *C. C. Chesterman.*

A New Sulpha Compound ("6257") and Its Use in Human Cholera Infection. BHATNAGAR, S. S., FERNANDES, F., DE SA, J., and DIVEKAR, P. V. (1948). *Nature, Lond.*, **161**, 395.

Chemotherapy of Cholera with a New Sulphonamide Compound ("6257"). Laboratory Investigations and Field Trials. BHATNAGAR, S. S., FERNANDES, F., DE SA, J., and DIVEKAR, P. V. (1948). *Brit. med. J.*, **1**, 719.

Hexamethylene tetraamine was found to kill cholera vibrios suspended in physiological saline in less than half an hour. Crude preparations consisting of sulphanilamide and hexamines were first prepared and found to give promising results in animals and man infected with cholera. Later a compound ("6257") was prepared by condensing two molecules of sulphathiazole and three molecules of formaldehyde. *In vitro* it exerts a bactericidal action on cholera vibrios when 50 mg. or more is added to 10 ml. of peptone water; in concentrations of from 5 to 50 mg. in 10 ml. it is bacteriostatic. *In vivo* in mice inoculated intraperitoneally with cholera vibrios complete protection is afforded by doses of 40 to 50 mg. given subcutaneously or intraperitoneally for 2 days before inoculation and for 4 days after. If given by mouth only 10% of mice are protected, probably because of poor absorption from the alimentary canal.

Field trials were carried out in villages in Southern India. After bacteriological confirmation of the diagnosis a dose of 6 g. was given by mouth followed by 4 g. 4 hours later. Two doses of 2 g. were given on the second and third days, two doses of 1 g. on the fourth day, and 1 g. for the next 3 days. The usual total dose was 28 g., though as much as 50 g. has been given without any toxic results. If vomiting was severe the first doses were given by rectum. Among 85 patients, mostly undernourished women and children, the mortality has been 4%, whereas in the same area the average mortality for the past seven years has been 70%. Although no details are given the administration of the drug to healthy contacts is said to have protected them against infection.

G. M. Findlay.

Treatment of Epidemic Typhus with Chloromycetin. PAYNE, E. H., KNAUDT, J. A., and PALACIOS, S. (1948). *J. trop. Med. Hyg.*, **51**, 68.

Chloromycetin is an antibiotic obtained by Ehrlich *et al.* (*Science*, 1947, **106**, 417) from an actinomyces isolated from the soil of a field in Venezuela. It has been found

by Smadel and Jackson (*Science*, 1947, **106**, 418) to be active against psittacosis virus and against a number of rickettsiae in mice. It has now been used on 16 patients in Bolivia with epidemic louse-borne typhus. The antibiotic may be given intravenously or by mouth, the former giving more rapid results. Intravenous medication consists of doses of 10 mg. per kilo body weight for 3 days, while by mouth at least 15 mg. per kilo of body weight may be given without toxic reactions for the same period. *G. M. Findlay.*

Observations on the Action of Paludrine on Malarial Parasites. MACKERRAS, M. J., and ERCOLE, Q. N. (1947). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 365.

When "paludrine" was given to patients infected with *Plasmodium vivax* a lethal action was exerted upon the dividing nucleus of the malaria parasite, which is rapidly destroyed. Paludrine does not directly prevent the formation of gametocytes or destroy those already formed; it does not prevent fertilization in the gut of the mosquito. Similar effects are observed with *P. falciparum*. In the presence of paludrine gametocytes can be formed; they persist in the blood, undergo fertilization in the mosquito, and reach the stage of encystment, but development ceases at that point. Paludrine exerts a similar effect on the early schizonts of *P. malariae*, although these are somewhat less sensitive than those of the other two species of malaria. *F. Hawking.*

Bacteriology and Biology of Disinfection of Bile, with Special Reference to Pyridine-3-carbonyl-hydroxymethylamide (Zur Bakteriologie und Biologie der Gallendesinfektion, unter besonderer Berücksichtigung des Pyridin-3-karbonsäureoxymethylamids). ACKLING, O. (1948). *Praxis*, **37**, 65.

Pyridine-3-carbonyl-hydroxymethylamide ("bilamid") is a new chemical compound which can be regarded as nicotinic acid amide linked to formaldehyde.

Nicotinic acid plays an important part in the metabolism of the liver, and this new compound exerts its action on the liver and bile. The nicotinic acid component promotes the normal metabolic activities of the liver cells while the formaldehyde component acts as a disinfectant on organisms present in the bile ducts and gall-bladder. The results of animal tests are given showing that when given intermittently for several consecutive days a total daily dose of 2 g. per kilo causes no symptoms, while 4 g. per kilo represents the minimal lethal dose for this scheme of medication. Maximal concentrations of bilamid in the bile are obtained 2 hours after an oral dose of 2 g. per kilo or half an hour after 4 g. per kilo. Within the following 4 to 6 hours the level falls rapidly but traces are still present after 24 hours. Excretion, therefore, is rapid and complete. The drug is also excreted in the urine and can be demonstrated there 1 hour after an oral dose of 4 g. per kilo. The urine content steadily increases until 3 to 4 hours after administration; no bilamid is present in urine after 10 hours.

Bilamid has a bactericidal action on most strains of the coliform group, including organisms causing enteric fever, on *Streptococcus faecalis* and *Pneumococcus* strains, on *Brucella abortus*, and on the cholera vibrio. Bacteriostatic but not bactericidal actions of bilamid were recorded for dysentery, coli, anthrax, and pyocyaneus bacilli as well as for staphylococci.

Figures and tables are given to demonstrate the action of the drug on the flora found in specimens of bile.

K. Zinnemann.

The Treatment of *B. coli* Urinary Infections with Sulfathalidine (Phthalylsulfathiazole). EVERETT, H. S., VOSBERG, G. A., and DAVIS, J. M. (1948). *J. Urol.*, **59**, 83.

As the result of a controlled series of cases the author has concluded that phthalylsulphathiazole is an effective drug in the treatment of urinary tract infections due to *B. coli*. The urine is usually rendered sterile in 1 week by the administration of 0.1 g. per kilo body weight daily. Experience has shown that continuation of the treatment for an additional 2 weeks gives greater protection against recurrence. The author suggests that the good results are due mainly to the action of the drug in eliminating in the bowel the source of infection of the urinary tract.

Thomas Moore.

Observations on the Prevention of Bacterial Growth by Sulphonamides, with Special Reference to the Harper and Cawson Effect. WALKER, N., PHILIP, R., SMYTH, M. M., and MCLEOD, J. W. (1947). *J. Path. Bact.*, **59**, 631.

The phenomenon of inhibition of staphylococci and streptococci by horses' blood in an area adjacent to a sulphathiazole trench, known as the Harper and Cawston effect (*J. Path. Bact.*, 1945, **57**, 59), has been more fully investigated by the present authors in an endeavour to find an accurate explanation. Many types of blood have been examined: only asses' blood has anything like the same power as horse blood. Beyond revealing the fact that the property appears to be in the haemoglobin fraction, further analysis has not been helpful.

The Problem of Sulfonamide-resistant Hemolytic Streptococci. HARTMAN, T. L., and WEINSTEIN, L. (1948). *New Engl. J. Med.*, **238**, 560.

The authors have studied 167 patients suffering from Group A haemolytic streptococcal infections. Most of the cultures were isolated from the nasopharynx of patients who had clinical scarlet fever. The type distribution of these haemolytic streptococci and their sulphonamide sensitivity were studied. Only one strain, a Group A, Type 19, was resistant to the action of sodium sulphadiazine, and was so in a concentration of 25 mg. per 100 ml. The epidemic Type 19 strains prevalent among Service personnel were resistant to a similar concentration of sulphadiazine.

R. N. Johnston.

Nose and Skin Carriage of *Staphylococcus aureus* in Patients Receiving Penicillin. MOSS, B., SQUIRE, J. R., and TOPLEY, E. (1948). *Lancet*, **1**, 320.

Numerous publications have described the presence of *Staphylococcus aureus* in the nasal cavities of a high percentage of healthy people. The presence of the same organism, defined by a positive coagulase test, is of less frequent occurrence on the healthy skin. The authors in their present study have concentrated on the specific problem of the dependence of skin carriage on nasal carriage in persons with normal skins. Some of the patients in a male surgical ward were the subjects of the investigations over a period of 8 months. Penicillin was given intranasally for 10 days: (1) by spraying thrice daily with a solution containing 12,500 units per ml., and (2) by applying a cream containing 100,000 units per g. in a "Lanette wax" base with sterile swabs twice daily. The details of the bacteriological technique are given.

In a group of 21 patients the nasal-carriage rate fell from 97% before treatment to 67% during the first 5 days of treatment and to 37% during the last 5 days, whereas in a control group of 20 the bacterial flora of the nose showed no significant change as a result of their stay in hospital. The elimination of the nasal *Staph. aureus* was associated with a significant fall in its carriage on the skin of the wrist, and this would seem to show that the skin is often contaminated from the nose. In 37 cases swabs were obtained from the vestibule, nasal fossae, and nasopharynx, and the findings emphasize the decreasing frequency with which the staphylococcus is found at sites from the nasal vestibule to the nasopharynx, and that colonization seems to take place only in the squamous epithelium of the vestibule. In 15 cases parenteral administration of penicillin did not affect the incidence of staphylococci in the nose. *J. Smith.*

Penicillin Therapy in Diphtheria. (In English.) WSZELAKI, S. J., and HANDZEL, L. J. (1948). *Acta med. scand.*, **129**, 493.

The authors' proposals for treatment are as follows: (*a*) In the early case penicillin alone may be given, if there is adequate opportunity for clinical observation of the course of the illness. Penicillin may also be used alone where serum treatment is contraindicated—the authors list as examples, active pulmonary tuberculosis, hypersensitization, and "grave blood diseases." (*b*) Combined penicillin and serum treatment is indicated in a penicillin-sensitive concomitant infection, in very severe but uncomplicated diphtheritic infections and late cases, and in cases "refractory" to serum treatment. A sufficient dose of penicillin is 100,000 units a day.

[It may be noted that simple penicillin treatment in certain cases of diphtheria has been recommended by De, Chatterjee, and Ganguli (*Brit. med. J.*, 1947, **1**, 376) and strongly condemned by Long (*Brit. med. J.*, 1947, **1**, 884), who has suggested that 1,000,000 units of penicillin daily may be advantageously combined with serum treatment in severe cases.] *G. I. C. Ingram.*

Effect of Penicillin on the Bacteremia Following Dental Extraction. GLASER, R. J., DANKNER, A., MATHES, S. B., and HARFORD, C. G. (1948). *Amer. J. Med.*, **4**, 55.

The authors report their observations in 40 patients who received 50,000 units of penicillin intramuscularly 2-hourly for 24 hours before the extraction, and compare the results with those obtained in controls who did not receive penicillin. Blood for culture was taken before and usually within 2, but always within 5, minutes after the extraction. Penicillin reduced the incidence of bacteriaemia considerably, but failed to prevent it in all cases; the organisms grown were in both series α-haemolytic streptococci and non-haemolytic streptococci. The difference between the penicillin series and the control series was greatest in the groups with diseased gums, where the incidence of bacteriaemia was 41.4% (penicillin) and 78.9% (controls). A pronounced difference in favour of penicillin was also seen in the group of single extractions; with multiple extractions the percentages were equal in both series. Local analgesia was used in all cases; the effect of penicillin was greater, comparatively, in the group who had infiltration analgesia than in those who had block analgesia. The authors recommend that patients suffering from valvular or congenital heart disease should receive large doses of penicillin for 24 hours before and at least 2 days after extractions, which should preferably be carried out singly. *B. Samet.*

Penicillin in the Treatment of Actinomycosis. NICHOLS, D. R., and HERRELL, W. E. (1948). *J. Lab. clin. Med.*, **33**, 521.

The authors record the results of treatment of 46 cases of actinomycosis with penicillin. The dose varied from 80,000 to 1,000,000 units of penicillin daily (intravenously or intramuscularly) for periods of from 2 to 7 weeks. They conclude that a dose of at least 500,000 units should be administered daily for at least 6 weeks. "Penicillin appears to be an effective chemotherapeutic agent in the treatment of actinomycosis and a useful adjunct to other forms of therapy." *Zachary Cope.*

Penicillin Preparations with Delayed Excretion in Ophthalmology. (La penicilina de eliminación retardada en oftalmologia.) MATA LOPEZ, P. (1947). *Arch. Soc. oftal. hisp.-amer.*, **7**, 1111.

The disadvantage of penicillin is its rapid excretion; a further disadvantage in ophthalmology is that penicillin, when given intramuscularly, does not penetrate into the posterior parts of the eye. Various methods have been devised for delaying excretion of the drug, such as combining penicillin and blood plasma, cooling the site of injection, and using a wax-oil base for the penicillin. The author has found this last method most satisfactory and has used 3 such types of preparation, 100,000 units being given daily in two doses, one at night and the other in the morning. As an alternative an aqueous solution of penicillin may be given 3-hourly during the day and the emulsion of penicillin at night.

The author uses penicillin for persistent staphylococcal infections of the eyes, abscesses of the lids, dacryocystitis (local and general injections), gonococcal infections of the conjunctiva and cornea (combined with local application), infections of the cornea, certain cases of episcleritis, and uveitis. He has had good results with this method, which he has also employed prophylactically in cases of trauma. *E. E. Cass.*

Bacteriologic Follow-up of Penicillin-treated Gonorrhea in Women. HIRSCHBERG, N. (1948). *Amer. J. Syph.*, **32**, 141.

The author took the opportunity of observing the results of penicillin therapy over a post-treatment period of 10 weeks in women imprisoned for prostitution. Cases were selected for study only after the isolation and identification of the gonococcus by culture and fermentation tests with specimens from the urethra and cervix. All 54 cases were treated with 150,000 units of penicillin in oil-wax. There were only 2 failures of this treatment. In one the gonococcus was found again 3 weeks after treatment, and again 2 weeks after a second treatment. In the other case a positive culture was not obtained until 7 weeks after treatment. The author considers that cultures at regular intervals after apparent cure are of considerable importance. He also comments upon the larger number of Gram-negative bean-shaped diplococci which might be confused with the gonococcus in stained smears or in cultures when the results are unconfirmed by fermentation tests. *V. E. Lloyd.*

Mycotic Vulvovaginitis and the Vaginal Fungi. A Report of 280 Patients. JONES, C. P., CARTER, B., THOMAS, W. L., ROSS, R. A., and CREADICK, R. N. (1947). *Amer. J. Obstet. Gynec.*, **54**, 738.

The authors state that there are three groups of yeast or yeast-like fungi which may be found in the vagina—*Monilia*, *Cryptococcus*, and *Saccharomyces*. These organisms can only be distinguished by the morphological characteristics of colonies grown on special media. *Monilia*, or *Candida* (the name now recommended), is probably the only organism of this type which gives rise to symptoms. The clinical findings and diagnosis are described, and the methods of collecting and culturing specimens from patients are given. Intradermal skin tests and agglutination reactions are of little value in diagnosis. For treatment, a vaginal jelly with a "bentonite" base containing calcium and sodium propionate has given excellent results. *Braithwaite Rickford.*

Cultural and Serologic Studies on Granuloma Inguinale. DUNHAM, W., and RAKE, G. (1948). *Amer. J. Syph.*, **32**, 145.

After many unsuccessful attempts to grow *Donovania granulomatis* on artificial media, material from the ninety-second egg passage was successfully cultivated by incubation at 35° C. for 1 to 2 weeks after planting on slants consisting of 10 ml. of beef-heart infusion agar with 1 ml. of normal yolk from six-day embryos. Eight serial transfers were made with this medium and also to slants prepared from 5 ml. of 3% agar in tryptone beef-heart infusion broth and 5 ml. of modified Levinthal's stock broth. Colonies were irregular in outline, shiny, translucent, and grey. An antigen was prepared for serological studies by washing and suspending in M/100 phosphate buffered physiological saline, shaking and centrifuging, and employing the supernatant fluid, which was preserved in 1 in 10,000 "merthiolate." When complement-fixation tests were carried out with this as the antigen, 50 positive reactions were obtained from 58 sera of patients with granuloma inguinale, from 1 of 32 with syphilis, from 2 out of 18 with gonorrhoea, from 2 of 7 with lymphogranuloma venereum, from 3 of 10 with chancroid, and from 4 of 19 with varicose ulcers. No positive reactions were obtained from the sera of 10 patients with tuberculosis or of 4 normal persons.
R. R. Willcox.

Cardiolipin Antigens in Serologic Tests for Syphilis. GIORDANO, A. S., CULBERTSON, C. S., and HIGGINBOTHAM, M. W. (1948). *Amer. J. clin. Path.*, **18**, 193.

Cardiolipin Blood Tests in Syphilis. ANDUJAR, J. J., ANDERSON, M. M., and MAZUREK, E. E. (1948). *Amer. J. clin. Path.*, **18**, 199.

The theme of these articles is that cardiolipin is an advance on all other antigens, but that the best use has not yet been made of it. In the first paper 24,085 tests were examined, and in the second paper 24,609. It is concluded that cardiolipin antigens are highly sensitive and satisfactorily specific, and have the advantage of being stable and yielding a reproducible emulsion. Sponsored universal tests based on cardiolipin are urged.

The Incubation Period of Ophthalmia Neonatorum. SORSBY, A. (1947). *J. Obstet. Gynaec. Brit. Emp.*, **54**, 842.

The principal causal organism in ophthalmia neonatorum is now considered to be *Staphylococcus aureus* rather than the gonococcus, although the virus of inclusion blennorrhoea may be almost as common. A series of 290 cases of ophthalmia neonatorum were investigated. In 179 cases organisms only were found, in 67 both organisms and inclusion bodies, in 29 inclusion bodies only, and in 15 neither. Detailed figures are given of the distribution of incidence according to the causal organism; in general, bacteria appeared in the first 5 days and viruses in the second 5. The gonococcus causes the highest proportion of severe cases, and the diphtheroids the next highest; the inclusion bodies lie midway between these and the staphylococci.
Hugh R. Arthur.

BIOCHEMISTRY

The Value of the Guterman Test in Threatened Abortion. BENDER, S. (1947). *J. Obstet. Gynaec. Brit. Emp.*, **54**, 783.

This paper is a preliminary report on 55 cases diagnosed clinically as of threatened abortion, in which the Guterman test was carried out. The test was found to provide a rapid and reliable means of differentiating those cases of threatened abortion accompanied by progesterone deficiency from those not so accompanied. Experimental work is reviewed in support of the contention that the administration of progesterone where there is no deficiency of the hormone increases the chance of the abortion progressing. The results of the present investigation support this view, although the figures are too small to be statistically significant. The limitation of progesterone therapy to cases of threatened abortion with evidence of progesterone deficiency would increase the foetal salvage rate. It is pointed out that clinically it may be difficult to distinguish between cases of threatened abortion and those of missed and complete abortion and of corpus luteum cysts. The results of pregnanediol and gonadotrophin tests simultaneously performed are of value in reaching the correct diagnosis in these cases.
R. L. Hartley.

Investigations into the Determination of Pregnanediol According to the Guterman Method. KULLANDER, S. (1948). *J. Obstet. Gynaec. Brit. Emp.*, **55**, 159.

The author compares critically the methods of estimation of pregnanediol in urine. He reports on the results of 430 tests made on specimens collected from 338 patients, and arrives at the following conclusions. The variations in the day-to-day excretion of pregnanediol in non-pregnant and pregnant women are so considerable that the test should always be carefully correlated with the clinical picture, and conclusions should not be drawn from one test. In a normal menstrual cycle pregnanediol is excreted in greatest quantities in the luteal phase. The lowest excretion is during the proliferative phase. The high level associated with the luteal phase may be interpreted incorrectly as indicating the presence of cyesis, and for this reason the author believes that the Guterman test is not a reliable pregnancy test. Conflicting results have been recorded on the value of pregnanediol excretion as a guide to prognosis in threatened abortion; the author concluded that the test was unreliable for this purpose. In carcinoma of the adrenal the excretion of pregnanediol is high; in both hydatidiform mole and theca lutein cystadenomata the excretion is low. The test may be of value, therefore, in the diagnosis of these conditions. *J. Stallworthy.*

A Simplified Method for the Quantitative Determinations on Free Pregnanediol Excretion in Pregnancy. DAVIS, M. E., and FUGO, N. W. (1947). *Proc. Soc. exp. Biol. N.Y.*, **66**, 39.

Over 1,500 determinations of pregnanediol excretion have been carried out on a series of about 100 women. The method is an adaptation of that described by Guterman (*J. clin. Endocrinol.*, 1945, **5**, 407). The authors demonstrate the accuracy of their method by gravimetric comparisons.

Pregnancy Test using the Male Toad. MAININI, C. G. (1947). *J. clin. Endocrinol.*, **7**, 653.

A new biological test for pregnancy is described. The authors inject 10 ml. of first morning urine, untreated, into the lateral lymph sac of the toad, *Bufo arenarum*. Urine (1 or 2 drops) is taken by pipette from the urinary bladder of the toad and examined microscopically. The appearance of spermatozoa 3 hours after the injection is evidence of a positive reaction.

Results of Administration of Anterior Pituitary Adrenocorticotropic Hormone to a Normal Human Subject. MASON, H. L., POWER, M. H., RYNEARSON, E. H., CIARAMELLI, L. C., LI, C. H., and EVANS, H. M. (1948). *J. clin. Endocrinol.*, **8**, 1.

Pituitary adrenocorticotrophin, 25 mg., was given as a single dose to over 100 normal individuals and patients. The maximum effect was observed after 4 hours. The most pronounced change was a fall in the number of eosinophils in the normal subjects (167 to 40 per c.mm.) and in patients without Addison's disease (181 to 57 per c.mm.); this did not occur in 30 patients with Addison's disease (247 to 235 per c.mm.). The lymphocyte count also decreased, but not to the same degree, and there was a slight increase in the number of the neutrophil cells. The lymphocyte response was absent in the Addisonian cases, but the increase in the number of neutrophils was present. The eosinophil and lymphocyte response in those with Addison's disease, although absent after adrenocorticotrophin, could be elicited with 17-hydroxycorticosterone (20 mg.), but not with desoxycorticosterone and dehydrocorticosterone hemisuccinate. The very striking difference in the eosinophil response is regarded as a reliable clinical test for the adrenal-cortical reserve of 11-17-oxysteroids in Addison's disease.

H. Herxheimer.

Determination of the Relative Activities of Anti-thyroid Compounds in Man Using Radioactive Iodine. STANLEY, M. M., and ASTWOOD, E. B. (1947). *Endocrinology*, **41**, 66.

The rate of iodine uptake by the thyroid was measured by giving a dose of radioactive iodine to normal volunteers and measuring the concentration in the gland. The concentration rose slowly during the period of absorption and then rapidly and parabolically to reach a constant level after 24 to 48 hours. Thirty-two compounds were thus tested in doses of 5 to 500 mg. The results obtained did not agree with the results of determinations of thyroid enlargement in rats caused by the drugs. For example, the relative activities of thiouracil, 6-*n*-propylthiouracil, thiourea, and 2-aminothiazole were 100, 1,100, 12, and 10 in the rat experiments and 100, 75, 100, and 250 in human studies. The results of the tests on human subjects agreed better with clinical experience, but discrepancies still remain. These are perhaps due to cumulative action, which cannot be assessed by this one-dose method of testing. *Peter C. Williams.*

Metabolic Studies in Diabetic Acidosis. II. The Effect of the Administration of Sodium Phosphate. FRANKS, M., BERRIS, R. F., KAPLAN, N. O., and MYERS, G. B. (1948). *Arch. intern. Med.*, **81**, 42.

Previous workers have shown that in diabetic acidosis the inorganic phosphorus level in plasma is raised, but falls rapidly when insulin is given. This is confirmed by the authors in a series of 28 patients in whom the plasma phosphorus ranged from 4.23 to 17.2 mg. per 100 ml., and fell to less than 3 mg. per 100 ml. shortly after the start of insulin therapy. This decrease was not due to a more rapid excretion, for the phosphorus concentration in urine became subnormal, and remained so for up to 5 days, while there was a dramatic fall in plasma phosphorus in one patient with complete anuria. Since, in uncontrolled diabetes, the phosphorus concentration in urine is known to be raised, these observations suggested that in diabetic acidosis the phosphorus stores of the body are depleted, with a resulting deficiency of available phosphorus.

The authors suggest that in diabetic acidosis the lack of insulin results in a failure of phosphorylation of glucose, and so causes an accumulation of inorganic phosphorus in the plasma, and an increased excretion in the urine. They cite the observation that in alloxan diabetes in rats the onset of coma is accompanied by a decrease in the organic phosphates of the liver (chiefly adenosine diphosphate and triphosphate) and an increase in the plasma inorganic phosphorus. When insulin is given the process of phosphorylation is resumed, and for the reconstitution of organic phosphates an increased intake of phosphorus is necessary. It is suggested, therefore, that the parenteral administration of sodium phosphate should be included in the treatment of diabetic coma 4 to 8 hours after the first dose of insulin.

Wilfred E. Hunt.

Some Factors Affecting the Acidity of Urine in Man. EGGLETON, M. G. (1947). *J. Physiol.*, **106**, 456.

An increase in the urine acidity (hydrogen-ion concentration) may be due not only to increased output of hydrogen ions but also to a reduction in buffering power. In experiments on 30 to 40 subjects the *p*H, ammonia concentration, and buffering power of urine were measured after the ingestion or intravenous injection of certain substances. An increased acidity of the urine accompanied by an increase in buffer output, two-thirds of which is due to phosphate, follows the ingestion of ammonium sulphate or chloride. Intravenous injections of hypertonic sucrose or sodium sulphate cause a rise in hydrogen-ion concentration and a fall in buffer output, whereas the ingestion of hypertonic urea causes a fall in hydrogen-ion concentration and a rise in buffering power. Diuresis gives rise to an increased output of buffer substances which masks the inverse relation between hydrogen-ion concentration and buffer output. The hypothesis is advanced that the increase in urine acidity, fall in buffer output, and relatively small diuresis which follow the injection of sodium sulphate and sucrose are due to secretion of antidiuretic hormone.

E. F. McCarthy.

Further Observations on the Significance of the Blood Pyruvic Acid Level in Infancy. ALLIBONE, E. C. (1948). *Arch. Dis. Childh.*, **23**, 7.

The author thinks that the rise in blood pyruvic acid in toxaemic and haemolytic states during infancy is not associated with a deficiency of thiamine and is probably unrelated to any of the known factors in the vitamin-B complex.

The Prothrombin Response to the Parenteral Administration of Large Doses of Vitamin K in Subjects with Norman Liver Function and in Cases of Liver Disease : A Standardized Test for the Estimation of Hepatic Function. UNGER, P. N., and SHAPIRO, S. (1948). *J. clin. Invest.*, **27**, 39.

The authors describe a new standardized test for the estimation of hepatic function depending on the prothrombin response to administration of large doses of vitamin K. The test was applied in 113 patients, 57 without and 56 with clinical evidence of liver disease. In the latter group 45 gave a positive response to the test, 6 a doubtful response, and 5 a negative response. The authors consider the method an important addition to the range of tests of hepatic function, since it deals with another activity of the liver. *J. W. McNee.*

Liver Involvement in Infectious Mononucleosis. EVANS, A. S. (1948). *J. clin. Invest.*, **27**, 106.

Liver function tests were carried out in 19 cases of infectious mononucleosis. Seventeen of the patients had agglutinins for sheep cells in dilutions of 1 in 160 or higher, while of the 2 remaining patients, 1 with agglutinins at a titre of 1 in 80 had antibodies with the guinea-pig kidney and beef red cell absorption characteristics of the infectious mononucleosis antibody, and the other had a typical blood picture and clinical features.

The cephalin-cholesterol flocculation test was abnormal in 95% of the cases of infectious mononucleosis, and in 2 instances had altered before the heterophil antibody titre had reached a significant level. In the 2 cases in which there was a normal flocculation test, illness was mild and the patients remained ambulatory. Raised values for the thymol-turbidity test were found in 68% of cases, ranging from 4 to 10 units. Abnormalities in this test usually appeared later and were more transient than the changes in the flocculation reaction. The serum alkaline phosphatase test showed increased values in 43% of the cases, while in no case was there a significant increase in the total serum bilirubin. Serum protein determinations were made in one case of infectious mononucleosis without jaundice and in one case with jaundice, the serum having been taken about 6 weeks after the onset of the illness in each case. Marked increases in the percentages of β-globulin and γ-globulin were observed in both.

The author concludes that hepatitis occurs in many cases of infectious mononucleosis without jaundice. The cephalin-cholesterol flocculation reaction is a more sensitive indicator of this hepatic dysfunction than is the thymol-turbidity test, and may be used in differentiating such cases from cases of uncomplicated upper respiratory infection. *R. B. Lucas.*

HAEMATOLOGY

Chronic Myelogenous Leukemia. A Study of 129 Cases in which Treatment was with Radioactive Phosphorus. LAWRENCE, J. H., DOBSON, R. L., LOW-BEER, B. V. A., and BROWN, B. R. (1948). *J. Amer. med. Ass.*, **136**, 672.

In this review the results, technique, and dangers of radio-phosphorus therapy are reviewed. There is considerable variation in sensitivity from patient to patient, and individual assessment of dosage is necessary so as to avoid overdosage and depression of the marrow. Life was not significantly lengthened by radio-phosphorus therapy, and at least one-third of the patients developed a terminal acute leukaemic phase. No other malignant tumours developed during the course of therapy.

Reflections on the Treatment of Acute Leucoses by Exsanguination Transfusions. (Réflexions sur le traitement des leucoses aiguës par l'exsanguino-transfusion.) BERNARD, J., and BESSIS, M. (1948). *Sang*, **19**, 45.

The effects of exchange transfusion in acute leukaemia are recorded. Both clinically and haematologically dramatic temporary improvement was obtained, with subsidence of enlarged lymph glands and spleen, and a tendency towards a more normal blood and marrow picture.

Course of an Acute Leucosis Treated by Repeated Transfusions and Penicillin. (Evolution d'une leucose aiguë traitée par les transfusions répétées et le pénicilline.) MAY, E., CATTAN, R., FRUMUSAN, P., and BILSKI-PASQUIER, G. (1948). *Rev. Hémat.*, **3**, 13.

The authors record the progress of a patient with acute leukaemia who was given 24 transfusions of about 150 ml. of blood during a period of 6 weeks. There was striking clinical and haematological improvement; the percentage of primitive leucocytes in the blood fell from 98% to 4% and the total leucocyte count from 40,000 to 6,400 per c.mm. The bone marrow obtained by sternal puncture was reported as normal. After a further 6 weeks the patient relapsed and died.

Remissions in Acute Leukaemia. (Les rémissions de la leucémie aiguë.) DREYFUS, B. (1948). *Rev. Hémat.*, **3**, 29.

The author has reviewed the literature dealing with remission in acute leukaemia, and concludes that in almost every case this has followed blood (or plasma) transfusions. He considers the possible mechanisms by which transfusions, including exchange-transfusions, may produce a favourable effect in the first instance.

The Skin Lesions of Monocytic Leukaemia. FAIRBURN, E. A., and BURGEN, A. S. V. (1947). *Brit. J. Cancer*, **1**, 352.

A case is reported with furunculosis as the first manifestation. The cutaneous lesions of 50 published cases of the Schilling type of monocytic leukaemia are then discussed and classified. Skin lesions were most frequently seen in men over 40 years of age.

Crystalline Vitamin B_{12}. RICKS, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., and FOLKERS, K. (1948). *Science*, **107**, 396.

The authors describe the isolation from liver of a red crystalline substance effective in the treatment of Addisonian pernicious anaemia when given in doses as small as 3 to 150 μg. (It is possible this substance (named Vitamin B_{12}) is the purified liver anti-anaemic factor. It is not yet known whether the neurological changes in pernicious anaemia are also controlled by it.)

Activity of Vitamin B_{12} for the Growth of *Lactobacillus lactis*. SHORB, M. S. (1948). *Science*, **107**, 397.

Liver extracts contain a growth factor (LLD factor) for *Lactobacillus lactis* in concentrations which closely parallel their anti-anaemic potency. The LLD factor is probably vitamin B_{12}.

Activity of Vitamin B_{12} in Addisonian Pernicious Anemia. WEST, R. (1948). *Science*, **107**, 398.

Satisfactory responses were obtained in 3 patients with Addisonian pernicious anaemia treated with simple injections of 3, 6, and 150 μg. of Vitamin B_{12} respectively.

The Reduction of Methaemoglobin in Red Blood Cells and Studies on the Cause of Idiopathic Methaemoglobinaemia. GIBSON, Q. H. (1948). *Biochem. J.*, **42**, 13.

This is an important paper. Idiopathic methaemoglobinaemia appears to be caused by an inborn deficiency of coenzyme factor I, a link in the chain of reactions whose effect is to prevent the oxidation of haemoglobin to methaemoglobin. The reducing action of ascorbic acid helps to prevent the complete oxidation of haemoglobin to methaemoglobin. Methylene blue is effective in treatment but reduces methaemoglobin by another enzymic pathway.

Refractory Iron-Deficiency Anaemia Treated with Intravenous Saccharated Oxide of Iron. DAVIDSON, L. S. P., and GIRDWOOD, R. H. (1948). *Brit. med. J.*, **1**, 733.

A good haematological response was obtained when a patient was treated with intravenous iron according to the method of Nissim (*Lancet*, 1947, **2**, 49), but there were some toxic reactions.

Chronic Haemolytic Anaemia with Haemoglobinuria. The Marchiafava-Micheli Syndrome. HICKEY, M. D., and MALLEY, L. K. (1948). *Quart. J. Med.*, **27**, 1.

The authors describe observations made on a patient observed for 3 years. Haemoglobinuria appeared to be precipitated on four occasions following iron therapy and once following blood transfusion. Normal human serum heated to 56° C. had some inhibiting effect on haemolysis both *in vitro* and *in vivo*.

Studies on the Pancytopenia of Kala-Azar. CARTWRIGHT, G. E., CHUNG, H. L., and CHANG, A. (1948). *Blood*, **3**, 249.

This is an important contribution to the haematology of kala-azar. The bone marrows of 27 patients were examined. There was general hyperplasia with increase in reticulo-endothelial cells. It is suggested that the anaemia, leucopenia, and thrombocytopenia are consequent on a disturbance in cell development, perhaps due to "hypersplenism," and are not simply due to a crowding out of the marrow cells by hyperplasia of the reticulum.

Studies on the Conglutination Test in Erythroblastosis Foetalis. WIENER, A. S., and GORDON, E. B. (1948). *J. Lab. clin. Med.*, **33**, 181.

The authors compared several techniques in the demonstration of Rh antibodies in the sera of pregnant Rh-negative women. The sensitivity of the tests in ascending order were: (1) the blocking test (the least satisfactory), (2) the plasma conglutinin and antiglobulin tests (Coombs' test), and (3) the albumin-plasma method (the most sensitive). Incomplete antibodies seem to pass through the placenta readily, but complete antibodies generally do not appear to do so.

Physiological Jaundice of the Newborn. Some New Measurement of the Factors Concerned. MOLLISON, P. L. (1948). *Lancet*, **1**, 513.

This is an important contribution to the understanding of the physiological jaundice of the newborn. Using the method of differential agglutination, transfusion studies carried out with placental and adult corpuscles demonstrated that a proportion of the infant's corpuscles are broken down at about twice the adult rate during the first 10 days of life. There is thus some indirect evidence of an increased rate of haemoglobin catabolism in the neonatal period. Previous work and the author's own observations of impaired bromsulphalein excretion by the infant's liver suggest, however, that inefficiency of the liver is the most likely cause of neonatal jaundice.

Congenital Hemolytic Jaundice. The Pathogenesis of the "Hemolytic Crisis." OWREN, P. A. (1948). *Blood*, **3**, 231.

The author demonstrates how "anaemic" crises may be produced during the course of congenital haemolytic jaundice. Six patients were studied and a temporary depression of erythropoiesis was found. Serial bone marrow studies demonstrated how restoration of erythrocyte formation preceded clinical recovery. These observations are of great interest and prompt the question as to how many of the so-called "haemolytic crises" previously reported have a similar explanation.

Normal Red-cell Survival in Men and Women. CALLENDER, S. T., POWELL, E. O., and WITTS, L. J. (1947). *J. Path. Bact.*, **59**, 519.

The survival of transfused normal blood was contrasted in 2 men and 4 women. In the men the elimination curve was linear; in the women it was appreciably curved. In both, elimination was complete in about 120 days. The average life of the transfused corpuscles was 63 days in the men and 54 days in the women. Blood loss due to menstruation is thought partly to explain the differences between the two sexes.

Blood Volume in Pregnancy. A Critical Review and Preliminary Report of Results with a New Technique. MCLENNAN, C. E., and THOUIN, L. G. (1948). *Amer. J. Obstet. Gynec.*, **55**, 189.

The authors studied the blood volumes of 20 pregnant women at term and at 7 weeks post partum, and 10 non-pregnant controls. They used Evans blue dye. The total blood volumes and plasma volumes were significantly greater in the pregnant women, but individual variation was great and many more determinations need to be done before a reliable picture is obtained.

A Method for Obtaining Bone Marrow by Vertebral Spinous Process Puncture. HUSS, J. H., GILBERT, J., and LIEBOW, A. A. (1948). *Yale J. Biol. Med.*, **20**, 291.

The spines of the lower thoracic and upper lumbar vertebrae may be punctured safely and readily, and marrow is withdrawn similar in composition to sternal marrow. This method is also described by Loge (*Blood*, 1948, **3**, 198); it is probably safer and less alarming to the patient than is sternal puncture.

Study of Fixed Tissue Sections of Sternal Bone Marrow Obtained by Needle Aspiration. I. Method and the Morphology in Various Conditions. II. Comparison of Nucleated Cell Count and Volumetric Pattern with Histologic Appearance. WEISBERGER, A. S., and HEINLE, R. W. (1948). *Amer. J. med. Sci.*, **215**, 170.

In this report are described a technique for sectioning marrow particles obtained by aspiration and a comparison between the total nucleated cell count, the volumetric pattern as determined by haematocrit, and the histological appearance of the marrows of 60 patients.

Clinical Value of Some Methods of Estimating Erythrocyte Sedimentation Rate. SINTON, J. R. (1948). *Brit. med. J.*, **1**, 391.

A comparison was made between the erythrocyte sedimentation rates (Westergren and Wintrobe techniques) of 61 female patients with pulmonary tuberculosis and the clinical course of the disease. The corrections of Wintrobe and of Whitby and Hynes for anaemia were applied. The patients, whose ages ranged from 14 to 46 years, were not specially selected, and were under continuous observation for periods ranging from 6 weeks to 6 months.

The author concludes that since the Westergren method gives figures that "agree better" with the clinical course than do those obtained by either of the methods involving correction, it is the most useful in assessing the degree of activity of the disease and the direction of its progress. *Wilfred E. Hunt.*

Erythrocyte Sedimentation Rate. The Effect of Alcohol as Contaminant. SYKES, W. O. (1948). *Brit. med. J.*, **1**, 393.

The rate of sedimentation is diminished if blood is contaminated with alcohol in concentrations of 1% or more.

MORBID ANATOMY AND HISTOLOGY

Phase-contrast Microscopy: Applications to Pathological Histology and Blood Cytology. (L'examen microscopique par les "contrastes de phases." Ses applications à l'histologie pathologique et à la cytologie sanguine.) FEISSLY, R., and QUÉBATTE, R. (1947). *Rev. Hémat.*, **2**, 411.

A simple explanation of the principles of phase-contrast microscopy, quite the most elementary which the abstracter has yet seen, and well worth studying.
G. Discombe.

Sludged Blood. KNISELY, M. H., BLOCH, E. H., ELIOT, T. S., and WARNER, L. (1947). *Science*, **106**, 431.

Histological observations were made on living animals and men; the authors claim that these lead to a more precise understanding of a variety of mechanisms whereby injuries and diseases damage the human body. In 600 non-anaesthetized human patients suffering from a wide variety of diseases the blood cells were seen to be agglutinated into masses (not rouleaux). This appeared to change the blood from its normal relatively fluid state to a circulating sludge. The authors believe that: (1) the resistance of sludged blood to its own passage through the bottle-necks of the circulatory system reduces the rate of blood flow through all the open vessels of the body; (2) agglutinated red cells are ingested and destroyed by the phagocytic cells of the liver and spleen; (3) there is settling and sedimenting of masses of agglutinated blood cells out of the moving blood plasma during life; (4) various degrees of reduction in circulating blood volume cause intermittent, prolonged, controlled shutting off of the arterioles in a selected series of tissues and organs.

The authors studied sludges in the conjunctival vessels in infective and traumatic conditions, hypertension, embolic states, hysteria, alcoholism, and normal uncomplicated pregnancy. Although it is not suggested that the sludges explain the clinical features of diseases in which they are found, they are obviously of importance in embolic conditions. The authors hope that it will become possible to keep the blood in a fluid and non-agglutinated state with intact vessel walls in conditions in which sludges have been observed.
R. Winston Evans.

Primary Systemic Amyloidosis. IVERSON, L., and MORRISON, A. B. (1948). *Arch. Path.*, **45**, 1.

Two cases of atypical, or primary, amyloidosis are reported in both of which the heart was extensively involved and many other tissues affected. Difficulties in the classification of amyloid disease and the staining properties of the substance in different circumstances are discussed.

Histological Changes in the Small Intestine in Disturbances of Fat Absorption. (Xanthomatosis of the Small Intestine.) (Über die histopathologischen Veränderungen am Dünnadarm bei Störungen der Fettresorption (sog. Xanthomatose des Dünndarms).) FREI, R. (1947). *Schweiz. Z. Path. Bakt.*, **10**, 685.

Two cases of xanthomatosis of the intestine are reported. A woman of 84 died of cerebral arteriosclerosis, apparently without having shown signs of gastro-intestinal upset during life. At necropsy the small gut showed yellowish discoloration of the rugae, and there was ulcerative colitis. Microscopically there was an extensive xanthomatosis of the submucous coat of the small intestine without signs of inflammation, and fulminating colitis was present in the large gut. The second patient, a man of 43, died after a sprue-like illness of several months' duration. On microscopical examination large foam cells were seen in the submucosa of the entire gut; in addition the lymph nodes of the mesentery showed fatty infiltration and a granulomatous xanthosis. *R. Salm.*

The Adrenal Cortex in Essential and Renal Hypertension. FISHER, J. A., and HEWER, T. F. (1947). *J. Path. Bact.*, **59**, 605.

Material was taken from routine necropsies on 55 subjects with essential hypertension, 15 with renal hypertension, and 57 controls. A diagnosis of hypertension was based on the presence of two out of three positive criteria: a high blood pressure record, left ventricular hypertrophy (measured by heart weight), and hypertensive arteriolar changes. In assessing heart weight the criteria of Moritz and Oldt were applied. Heart weights of 450 g. in males or 350 g. in females were taken to indicate hypertension, and those weighing less than 400 g. in males and 300 g. in females to denote absence of hypertension. Blood-pressure readings of 160/90 mm. Hg or more were considered to indicate hypertension at any age, and no case was accepted as a control if there was a record of a systolic pressure of over 140 mm. or a diastolic over 90 mm. Hg.

Formalin-fixed adrenals were weighed and portions analysed for percentage lipid. Frozen sections were stained with Scharlach R and examined under polarized light. The content of sudanophil lipid and anisotropic lipid in the three equal zones of the cortex (outer, middle, and inner thirds) was recorded in terms of arbitrary units: 0, ±, 1, 2, or 3. Adding the values for the three zones gave a value for lipid content; ±, indicating a trace, was taken as ½. A significant association was shown to exist between essential hypertension and each

of the following: cortical nodularity, percentage lipid (estimated chemically), cortical sudanophil lipid, and cortical anisotropic lipid.

The authors take the view that increased cortical lipid is an indication of altered endocrine activity. They suggest that their findings may all reflect variation in a single normal process by which the adrenal cortex maintains glomerular filtration, for example, by increasing the efferent glomerular arteriolar tone. *F. A. Langley.*

The Relationship Between Rheumatic Carditis and Subacute Bacterial Endocarditis. MACILWAINE, Y. (1947). *J. Path. Bact.*, **59**, 557.

Aschoff bodies in the myocardium of patients with infective endocarditis have been noted in many reports in the literature. The author reviews the evidence on their significance, and concludes that they do not represent a reaction to the bacterial infection, but that their presence is evidence of a rheumatic carditis. In the work here recorded, 34 cases of subacute, and 12 cases of acute, bacterial endocarditis were studied histologically; in every case in which Aschoff nodes were present an attempt was made to estimate their age. Histological criteria are given for the division into: under 2 months, 2 to 4 months, 4 to 6 months, and over 6 months. In the subacute group, 29 had Aschoff nodes, 5 did not; of the acute cases 7 had Aschoff nodes, 5 did not. In many cases crops of rheumatic lesions of 2 or even 3 different age periods were present. The estimated age of the Aschoff bodies corresponded to the clinical duration of the bacterial endocarditis in 64.7% of the subacute and in 25% of the acute cases. The author concludes that in the majority of cases of subacute, and in a smaller percentage of cases of acute, bacterial endocarditis the bacterial lesion is superimposed upon the site of an active rheumatic carditis.

Liver Disease in Johannesburg. Relation to Pellagra. GILLMAN, J., and GILLMAN, T. (1948). *Lancet*, **1**, 169.

The authors found that only 33 (12.6%) out of 261 Africans who died accidentally had normal livers. By contrast in a control group of 90 Europeans who died accidentally 61 (67.8%) had normal livers. The lesions in most of the abnormal livers in Africans could be classified on histological grounds into the four types already described by these workers as occurring among pellagrins. In these types the deposition of large quantities of iron is the chief feature and represents a profound metabolic disturbance. It is suggested that the large inert iron-containing molecules may complicate the primary metabolic disorder associated with liver disease, and thus prepare the background for the development of premature arteriosclerosis, cirrhosis of the liver, keloid formation, and reticulosis, all of which are known to be prevalent among Africans.

Christopher Hardwick.

Hepatic Alkaline Phosphatase: Histological and Microchemical Studies on Liver Tissue in Normal Subjects and in Liver and in Bone Disease. SHERLOCK, S., and WALSHE, V. (1947). *J. Path. Bact.*, **59**, 615.

The histological distribution of alkaline phosphatase in portions of liver obtained by aspiration biopsy (in many cases from serial aspirations) was studied by a modification of Gomori's technique. Increase in the enzyme in liver-cells and walls of sinusoids was invariable in acute hepatitis; other variations characterized obstructive jaundice and cirrhosis.

Automatism of the Nephron and its Histological, Physiological, and Pathological Aspects. (Automatismo nefronico e suoi aspetti isto-fisiopatologici.) MONTALDO, G. (1947). *Arch. ital. Anat. Istol. pat.*, **20**, 45.

Studies were made on the structure of the juxtaglomerular apparatus of the human kidney in health and disease, and on the anatomical and physiological relation of the glomerulus and tubule. The author advances the conception of "automatism of the nephron," basing his theory on the peculiarities of structure of the afferent arteriole, the epithelioid cells in the wall of the distal end of which are continuous with the special preglomerular cells of Zimmermann's cushion. The latter tissue fills the triangular space between the afferent and efferent vessels and the intermediate segment of the tubule (that portion joining the ascending limb of Henle's loop to the second convoluted tubule). It is continuous on one side with the ampulla and on the other is, of course, contiguous with the macula densa. Thus Zimmerman's "Polkissen" forms a bridge between these different parts of the nephron, and evidence is adduced that this tissue is sensitive both to pressure and to chemical stimuli. It is thought to be sensitive to the osmotic pressure of the fluid in the intermediate tubular segment, and swells or shrinks according to the degree of dilution or concentration of this fluid. When the urine is dilute, swelling of the cushion causes the glomerulus to contract in systole, with a consequent slowing in the rate of filtration and in the rate of passage of the filtrate through the tubule, and thus the concentration of the urine rises. The opposite also occurs, and the relations of filtration and absorption can be expressed algebraically. In this way automatic self-regulation is established in each single nephron, although the rate of filtration also remains dependent on such general factors as blood pressure, hormonal and nervous stimuli, and drugs. Moellendorf's "nephronic index" relates the size of the glomerulus to its corresponding tubule. *In vivo* this relation may vary between wide limits, and the author suggests the term "dynamic nephronic index" to relate the powers of concentration and dilution of the nephron. Those diseases of the kidney most typically associated with disturbance of the power of concentration (nephrosclerosis, chronic diffuse glomerulonephritis) show the most markedly selective changes in the self-regulating tissue of the nephron. When hyposthenuria or isosthenuria is present there is always pronounced alteration in the afferent arteriole, cushion, and ampulla of the surviving glomeruli. These glomeruli can no longer be influenced by the state of the intratubular fluid, and the filtration rate depends on the degree of patency of the afferent vessel and on the systemic blood pressure.

E. G. Sita Lumsden.

Intestinal Ulceration Due to Arterial Necrosis (Malignant Hypertension and Polyarteritis Nodosa). LE NAVASQUEZ, S., and FRENCH, E. B. (1947). *Guy's Hosp. Rep.*, **96**, 85.

Clinical and post-mortem records are reported of 2 patients, one with malignant hypertension and the other with polyarteritis nodosa, both presenting a clinical picture of predominantly intestinal disease. The first patient had a short history of abdominal pain and bloody diarrhoea, the second a more prolonged story of anaemia and steatorrhoea. The most striking pathological finding in both cases was the presence of extensive ulceration due to necrotizing arteritis of the small intestine. The histological appearances of the arterial lesions in

both cases were very similar. In the case of polyarteritis nodosa there was also complete disorganization of the mesenteric lymph nodes, which were the seat of extensive ischaemic necrosis and replacement fibrosis; this, together with the ulcerative destruction of the intestinal mucosa, was the cause of the sprue-like syndrome which was present during life. Another interesting feature of this case was the persistent lymphopenia (25 to 1,250 lymphocytes per c.mm.), accounted for at necropsy by the finding of extensive ischaemic necrosis of the splenic lymphoid tissue and by the above-mentioned mesenteric lymph-node destruction. *John R. Forbes.*

Massive Neoplastic Embolism. TILL, A. S., and FAIRBURN, E. A. (1947). *Brit. J. Surg.*, **35**, 86.

A tumour mass which had clearly originated in a pulmonary carcinoma was found in the common femoral artery, extending into the profunda artery with surrounding blood clot blocking these arteries and the superficial femoral artery.

The Nature of So-called Myoblastomata. (Die Natur der sog. Myoblastentumoren.) WEGELIN, C. (1947). *Schweiz. Z. Path. Bakt.*, **10**, 631.

Details of 10 cases of myoblastoma are given, the tumour being situated in gums, tongue, wrist muscles, and skin. The author thinks the growths are derived from undifferentiated mesenchymal cells and not from muscle elements and that they are capable of storing proteins and lipids.

Cancer of the Stomach in Addison's Anaemia. BOURNE, W. A. (1948). *Brit. med. J.*, **1**, 92.

The author investigated 15 unselected cases of pernicious anaemia, discovering 3 cases of carcinoma of the stomach and 1 of leiomyoma. In all 3 cases of carcinoma the neoplasm was in the lower third of the stomach, and radiology showed narrowing. This may be the first indication of carcinomatous change, or it may be a general characteristic of these stomachs. It is not productive of delay. The lower stomach is not usually abnormal in pernicious anaemia, and gastroscopy confirms this. The number of cases is too small for conclusions to be drawn, but the author suggests that in cases of pernicious anaemia an abnormal mucosa in the prepyloric region should be viewed with suspicion.

Intracranial Teratomas and Teratoid Tumors. (In English.) MULLER, R., and WOHLFART, G. (1947). *Acta psychiat., Kbh.*, **22**, 69.

In 8 cases of the rare teratoid form of cerebral tumour, 5 tumours were situated in the region of the pineal gland, 1 was in the third ventricle, 1 in the suprasellar fossa, and 1 in the cerebellum. Histological examination of the tumours revealed the presence of ectodermal and mesodermal structures in every case, but endodermal tissue was found in only 3 cases.

Carcinoma Ovarii and Cerebellar Degeneration. BROUWER, B., and SCHLESINGER, F. G. (1947). *Proc. K. Akad. Wet., Amst.*, **50**, 1329.

A patient, aged 51, with ovarian carcinoma, began to show cerebellar symptoms about 15 months before death. Psychical disturbances were present at the beginning but regressed. As in other reported cases the cerebrospinal fluid contained an excess of protein and gave colloidal reactions of mild paretic type. Post-mortem examination revealed carcinoma of the left ovary and adnexa with metastases in the neighbouring part of the abdominal cavity. The cerebellum showed no macroscopical evidence of disease, but microscopically there was complete disappearance of Purkinje cells, with relative preservation of baskets and almost complete integrity of the granular layer. The left dentate nucleus was severely degenerated, as were the ventral part of the right nucleus and to a minor degree the roof nuclei. No degeneration of inferior olives was seen. There was loss of many cells in the vestibular nuclei. Meningeal and perivascular infiltration in relation to the degenerated areas of the cerebellum and brain stem was present. The authors consider that the metabolic disturbances associated with carcinoma affect parts of the central nervous system which are predisposed to degeneration by abiotrophy in Gowers' sense of the word. *J. G. Greenfield.*

An Unusual Case of Adrenal Carcinoma with a Note on the Application of a New Colour Test. BROSTER, L. R., and PATTERSON, J. (1948). *Brit. med. J.*, **1**, 781.

A girl aged 14½ years had a history of increasing virilism for 18 months, and of fits followed by coma for 4. On admission she had right spastic hemiplegia (which passed in 3 days), bilateral papilloedema, hypoglycaemia, and a large left hypochondriac tumour. Radiographs showed calcified streaks in a large adrenal shadow and premature epiphysial fusion corresponding to 20 years or over. The 17-ketosteroid excretion was 1,980 mg. daily and the dehydro*iso*androsterone test (Patterson) was strongly positive. Death occurred a fortnight after admission, and 21 months after the onset of the virilism. At necropsy the left adrenal tumour together with the attached kidney weighed 6½ lb.; microscopically the structure was that of a malignant adrenal cortical tumour, although mitotic figures were scarce. No significant changes were found in other organs and there were no secondaries. Patterson's urinary colour test (*Lancet*, 1947, **2**, 580) was used to differentiate carcinoma from hyperplasia. *Henry Cohen.*

Tumors of the Carotid Body. LECOMPTE, P. M. (1948). *Amer. J. Path.*, **24**, 305.

This is a useful description of the characters of carotid body tumours, based on a personal study of 17 surgically removed specimens. No true chromaffin reaction was demonstrated in any of these, and no evidence of the secretion of adrenaline was obtained by assay of the fresh tissue in two instances. These findings thus support the other evidence that the carotid body is not part of the chromaffin system, but is a chemoreceptor. *R. A. Willis.*

Chemical Character of the Enterochromaffin Cells. GOMORI, G. (1948). *Arch. Path.*, **45**, 48.

The author discusses the histochemical reactions of the enterochromaffin cells and describes a new staining method (methenamine-silver) which is applicable to formaldehyde-fixed tissues. By using the Coujard technique he was able to show that the typical histochemical reactions of the granules are due to the presence of a resorcinol derivative. He has disproved the ideas of Lison and Cordier that a catechol compound exists, and he is unable to confirm the contention of Jacobson that a pteridine compound and desoxyribose are present in the cells. *E. T. Ruston.*

Papillary Carcinoma of the Thyroid and Lateral Cervical Region. So called "Lateral Aberrant Thyroid." CRILE, G. (1947). *Surg. Gynec. Obstet.*, **85**, 757.

A primary tumour was found in the thyroid gland itself in all of 16 consecutive cases in which the thyroid gland was explored or a lobe removed. In 4 of these the lesion was bilateral, but in none was more than a single nodule found in a lobe. The author reviews the difficult problem of the relationship of these papillary tumours to carcinoma of the thyroid in the light of personal experience.

Pathologists who have long taken the view that the lateral cervical masses were metastases of an unusually low degree of malignancy are thereby justified. Moreover it appears that the primary may remain small and easily escape detection from its frequent position in the posteromedial part of the lobe.

Lingual Goiter. Report of Three Cases. GOETSCH, E. (1948). *Ann. Surg.*, **127**, 291.

Three cases are described of hypertrophic thyroid tissue at the base of the tongue in non-migration of the thyroid anlage from the region of the foramen caecum. Each patient showed evidence of mild hyperthyroidism, and the histological structure of all three resected specimens was very similar to that of foetal adenoma of the cervical thyroid. It is pointed out that the majority of lingual thyroid swellings are goitres. These are benign, and carcinoma has been reported in only rare instances, always in men. Some pharyngeal and laryngeal obstruction is usually the indication for operation, and partial removal by the buccal route is the treatment of choice. Thyroid insufficiency is the rule after operation.

Anatomical and Clinical Study of Spontaneous Parathyroid Tetany in Adults. (Étude anatomo-clinique des tétanies parathyroïdiennes spontanées de l'adulte.) LEVRAT, M., and BRETTE, R. (1947). *Ann. Endocrinol., Paris*, **8**, 117.

Spontaneous parathyroid tetany (S.P.T.) has often been described in children, but only rarely in adults. A woman of 56 was treated for heart failure due to hypertension. She improved temporarily and remained under observation until her death 8 months later. Four months after her first admission she had an attack of S.P.T. which responded to calcium and to parathyroid extract, and best to combined calcium and vitamin-D treatment. She had had such attacks during the previous 2 years. Radiologically, cervical calcifications the size of a bean, which moved on swallowing, were found in the lateral thyroid regions. Necropsy showed the calcifications at the site of the parathyroids. Histologically, the parathyroids showed marked central sclerosis with calcium deposits and perivascular haemorrhage. At the periphery, parathyroid tissue was seen to be divided and encircled by fibrous tissue, as in cirrhosis of the liver.

Sarcoid. FREUDENTHAL, W. (1948). *Brit. J. Tuberc.*, **42**, 11.

This paper records evidence in favour of the belief that Boeck's sarcoid is a manifestation of tuberculosis. Certain links exist between the two diseases which do not, however, prove the hypothesis. Thus, coexistence occasionally occurs, skin sarcoid lesions have been known to change into lupus and *vice versa*, and terminal change of sarcoid into tuberculosis has been demonstrated at necropsy more than once. The chief factors against a tuberculous aetiology have been the histological picture, the negative tuberculin reaction, and the absence of tubercle bacilli. The author demonstrates sarcoid tissue in histological sections of lupus, and points out that it is formed in moderate amount during treatment with calciferol. Even more frequently, lupus features are seen in sarcoid. Lesions of spontaneous tuberculosis in some animals consist chiefly of epithelioid tissue, and in rats and dogs the tuberculin reaction remains negative. Wells and Wylie at Oxford have found that sarcoid serum actively neutralizes tuberculin. Tubercle bacilli have now been isolated from about 26 documented cases of sarcoid, and there is reason to believe that, as in tuberculides, bacilli can be demonstrated only at the earliest stage of the lesion. The newest finding in favour of a tuberculous origin is that sarcoid (presumably in skin) improves with calciferol therapy. *T. Semple.*

Primary Atypical Pneumonia. Report of Eight Cases with Autopsies. PARKER, F., JOLLIFFE, L. S., and FINLAND, M. (1947). *Arch. Path.*, **44**, 581.

Death in primary atypical pneumonia is uncommon. In the present 8 cases necropsy revealed congestion of the air passages and lungs, basal collapse with apical emphysema, and small grey pneumonic foci. Histologically, the alveolar exudate was "mononuclear"; there were proliferation and desquamation of alveolar epithelium and infiltration of septa and bronchioles. "Alveolar membranes" were present in half the cases. Many small arteries and veins were thrombosed. The lungs also showed changes due to secondary infection, such as pus in the bronchi. In the liver there were small focal necroses and in the brain, especially in one case, perivascular haemorrhages with glial proliferation. The appearances resembled those of psittacosis. In one case there were psittacosis antibodies, but this was probably fortuitous. Attempts to isolate a virus and demonstrate inclusion bodies were unsuccessful.

D. M. Pryce.

The Pathogenesis of Congenital Polycystic Lung and its Correlation with Polycystic Disease of other Epithelial Organs. Reconstruction of Cystic Elements in Two Cases. NORRIS, R. F., and TYSON, R. M. (1947). *Amer. J. Path.*, **23**, 1075.

The authors have written a series of articles on cystic disease in numerous organs and they discuss its pathogenesis, which might be a degenerative process leading to isolation and cyst formation, and may also involve the question of deficiencies of the circulation at the site of the cystic change. Two cases are here described of congenital cysts of a lung. Detailed anatomical descriptions showing lack of communication with the bronchi are given.

Pathology of Skeletal Muscle. (Zur Pathologie der Skelettmuskulatur.) HEDINGER, C. (1948). *Schweiz. med. Wschr.*, **78**, 145.

Two cases of damage to skeletal muscle after carbon monoxide poisoning are reported. In the first a contracture of the hand, of Volkmann type, took place; in the second a painful swelling of the gastrocnemius proceeded to calcification. Both cases were in young adults.

The author was able to trace 28 similar cases in the literature, and outlines the following entity. After severe

carbon monoxide poisoning there is transient albuminuria (rarely glycosuria), and in those parts of the body which have been exposed to pressure a triad may develop, consisting of skin damage and loss of sensibility and motility. The former resembles a burn. The sensibility is reduced distal to the skin lesions. Involvement of the muscles is shown by paralysis, swelling, and pain, and leads to permanent damage—paralysis, atrophy, contracture, calcification. Infection of gangrenous skin and underlying muscles may occur, with subsequent death. Biopsies show initially hyaline, fatty, or waxy degeneration of muscle, with some inflammatory reaction and occasional haemorrhage, and calcification and scar formation in the later stages.

Twenty-one Observations on Muscular Dystrophy in Hyperthyroidism. (Vingt et une observations de dystrophie musculaire hyperthyroïdienne.) FROMENT, R., GUINET, P., DEVIC, MME. M., and DEVIC, M. (1947). *Bull. Soc. méd. Hôp. Paris*, **63**, 843.

Of 21 cases investigated all except one were in women; in 16 toxic signs had existed for months, in the remainder for years. Weakness and difficulty in rising from a kneeling position were the chief muscular symptoms; wasting was found in most of the lower limb extensors and much less often in the arm muscles. Biopsies carried out in 3 cases revealed changes similar to those found in congenital myopathies. Creatinuria was present in 5 cases. Where the treatment of the hyperthyroidism was successful, the muscular dystrophy disappeared or was relieved, in one case after only 2 days but in most cases after months. Administration of iodine and aminothiazole, radium therapy, and thyroidectomy were the forms of treatment used. The authors speculate on whether the wasting is due directly to the thyroid state, or whether both run parallel for some unknown cause. Similar effects are seen in the muscles of animals after injection of thyroid extract. It is also emphasized that the two opposing states of thyrotoxicosis and myxoedema both produce muscular changes, though these are quite different in the two conditions; this suggests that the cause may lie in the thyroid itself. *T. E. C. Early.*

Talcum Powder Granuloma: A Frequent and Serious Postoperative Complication. EISEMAN, B., SEELIG, M. G., and WOMACK, N. A. (1947). *Ann. Surg.*, **126**, 820.

Thirty-seven cases of post-operative granulomata giving rise to clinical complications and proved due to talcum powder are described. It is stated that 22.6% of all gloves have perforations after use at operation. The risk of this post-operative complication is therefore considerable. Clinically the condition presents itself as a non-healing wound, a faecal fistula, intestinal adhesions, an obstruction or stenosis, or as a tumour mass. Talc produces pathologically the same kind of massive fibrous reaction as pulmonary silicosis, except that in the case of the granulomata the reaction is more localized. The usual histological picture of small round cells and multinucleated giant cells makes the resemblance to a tuberculoma obvious. The authors stress the danger of using talc and recommend that it be banned from surgery. Alternatives are discussed, the best proposed to date being potassium bitartrate.

Actinomycosis of the Brain. LEWIN, W., and MORGAN, A. D. (1947). *J. Neurol. Neurosurg. Psychiat.*, **10**, 163.

A case of actinomycosis apparently had its origin in the sphenoid bone, and also affecting the right cavernous, the superior longitudinal, the right sigmoid, and the superior petrosal sinuses, with extension to the right atlanto-occipital joint and the exterior of the right cerebral hemisphere. The patient was a man aged 31 years, and 5 months elapsed between the onset of symptoms and the necropsy, at which actinomyces were identified for the first time. Ventriculograms were normal, but there were significant alterations in the withdrawn fluid. From an abscess on the right side of the neck a non-haemolytic *Staphylococcus aureus* was grown. There was no evidence to show the route whereby the actinomyces reached the sphenoid. This is only the fourth recorded case of primary actinomycotic osteomyelitis of the sphenoid bone. *Zachary Cope.*

Studies in the Biology of the Cervix and its Relation to Puerperal Infections. MCILRATH, M. B., and HELLESTRAND, A. L. (1947). *J. Obstet. Gynaec. Brit. Emp.*, **54**, 746.

The authors feel justified in making the general conclusions that cervical lesions constitute a danger to the mother during pregnancy and increase her liability to develop low-grade pelvic infection after confinement. The added factor of cervical trauma does not seem to be of significance among primigravidae. On the other hand, among abnormal multigravidae the presence of pre-existing trauma and injury during labour does seem to aggravate a tendency to develop inflammatory conditions and to encourage spread to the uterus and adnexae. As a prophylactic measure, routine antenatal inspection of the cervix is recommended. When abnormal conditions are discovered the most satisfactory treatment appears to be the application of penicillin-sulphonamide powder.

[There are some good descriptions and illustrations of the histological changes which occur in the cervix during pregnancy.] *R. L. Hartley.*

Classification on the Histologic Reactions in Allergic Diseases. BOHROD, M. G. (1947). *Amer. J. Med.*, **3**, 511.

The author classifies the histological appearances of allergic reaction. Anatomically there are three main varieties: (1) necrotizing, which may be either organ-selective or cell-selective; (2) anaphylactoid; (3) granulomatous, which may be either tuberculoid or rheumatoid.

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SCIENCE AND THE BRITISH MEDICAL ASSOCIATION

The first of the two chief objects set out in the Memorandum of Association of the British Medical Association is the promotion of the medical and allied sciences, and it may fairly be said that pursuit of the second, namely the maintenance of the honour and interests of the medical profession, directly contributes to the attainment of the first. In the pursuit of its scientific activities, details of some of which are given below, the Association spends each year a considerable portion of its income.

Foremost amongst these activities is the publication of the *British Medical Journal*, with a weekly circulation of over 68,750 copies. The *Journal* serves as a comprehensive record of current developments and a forum for discussion in every field of medical science and professional organization in this country.

The Association also publishes monthly:

Abstracts of World Medicine, Abstracts of World Surgery, Obstetrics and Gynaecology,

and quarterly the following Specialist Journals:

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